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Birch Leaves as a Minefield: Do Trichomes Mediate Neonate Caterpillar Site Selection?

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ABSTRACT

Caterpillars are primary consumers that exert strong selective pressures on host plants. Plants, in turn, possess a range of defenses against herbivores. Leaf trichomes, for example, can act mechanically or chemically by damaging the insect's cuticle, restricting movement, disrupting attachment, or exposing insects to deterrent or toxic compounds. These defenses are especially challenging for neonate caterpillars, whose small size, soft bodies, and limited physiological capacity increase their vulnerability. To cope with these challenges, neonates have evolved multiple, non-exclusive strategies to evade or mitigate plant defenses. Here, we tested whether neonate caterpillars selected specific leaf sites in response to the distribution of non-glandular and glandular trichomes. We tested this with neonates of the masked birch caterpillar, *Drepana arcuata* Walker, 1855 (Drepanidae) on paper birch, *Betula papyrifera* Marshall (Betulaceae), leaves. We first quantified where neonate caterpillars established their shelter across leaf surfaces (adaxial vs. abaxial) and regions (base, middle, outer edge, and tip). Our findings showed that neonate establishment was non-random. Larvae more frequently established on the adaxial surface, even when initially placed abaxially, and predominantly near the leaf margins (base, outer edge, and tip) rather than in the middle. We then characterized trichome densities and tested whether they were associated with the distribution of established shelters. Both trichome types were non-uniformly distributed on paper birch leaves and negatively correlated with the number of established larvae, indicating that larvae preferentially selected sites with lower trichome densities. We also examined whether established larvae actively avoid feeding on trichomes. Larvae selectively bypassed glandular trichomes while skeletonizing leaf tissue. These results show that neonate *D. arcuata* selectively choose specific sites on leaves and that they avoid both non-glandular and glandular trichomes, suggesting that variation in leaf trichomes is an important determinant of micro-habitat selection in neonate caterpillars.

1 | Introduction

Caterpillars exert strong selective pressures on their host plants (Marquis and Koptur 2022), which in turn mount a range of defenses, including nutritional, chemical, and mechanical traits, that can modulate insect performance (Schoonhoven et al. 2005). Many plant defenses specifically target neonate (newly hatched) caterpillars, which represent one of the most vulnerable stages in the Lepidoptera life cycle due to their small size, poorly sclerotized cuticle, limited physiological capacity, and low mobility (Zalucki et al. 2002; Kaur et al. 2022).

To mitigate the challenges presented by plants, neonate larvae have evolved a suite of non-mutually exclusive morphological, physiological, and behavioral adaptations. Morphologically, features such as rigid mandibles, modified tarsi, and sclerotized prolegs are reported to enhance feeding efficiency and locomotion on plant surfaces (Hochuli 2001; Medeiros and Moreira 2002; Clissold 2007; Medeiros and Boligon 2007; Chowdhury et al. 2025). Physiologically, early instars possess detoxification mechanisms that allow them to process, and in some cases sequester, plant metabolites for defense (Musser et al. 2002; Li et al. 2002; Lahtinen et al. 2006; Weinhold and

Baldwin 2011; Jones et al. 2019). Behaviorally, neonates may reduce exposure to defenses by selectively feeding on less-defended tissues (Connor and Taverner 1997; Sinclair and Hughes 2010; Eaton and Karban 2014; Robertson et al. 2015), actively manipulating plant defenses by "mowing" trichomes or "sabotaging" leaf veins to disrupt toxic latex or resin flow (Clarke and Zalucki 2000; Shelomi et al. 2010; Hurley and Dussourd 2015; Robertson et al. 2015; Dussourd 2017), constructing silk mats or scaffolds that facilitate movement across structurally complex leaf surfaces (Rathcke and Poole 1975; Young and Moffett 1979) or feeding gregariously to collectively overwhelm defenses (Despland 2019; Qian et al. 2024). Another behavioral strategy for neonates is the selection of a specific initial establishment site on the host plant, either individually or in groups (Fordyce and Agrawal 2001; Watts and Kariyat 2021a).

Neonate larvae have been reported to select specific initial establishment sites on host plants; however, the patterns of within-leaf site selection, such as leaf surface (adaxial vs. abaxial) and position (base, middle, outer edge, or tip), vary widely among species (Casher 1996; Fordyce and Agrawal 2001; Horgan et al. 2007; Tagawa et al. 2008; Shelomi et al. 2010; Ang et al. 2014; Watanabe et al. 2018; Watts and Kariyat 2021a; Henault et al. 2022; Matheson et al. 2025; Tsuji and Ashford 2026). Many potential non-mutually exclusive factors may explain these patterns. Site selection may reflect larval preferences for leaf surfaces or regions with reduced epicuticular wax that enhance attachment and locomotion (Shelomi et al. 2010) or for younger, marginal, or softer tissues that improve feeding efficiency (Cockfield and Mahr 1993; Casher 1996; Ang et al. 2014). Other possible site attributes that could influence choice include those that facilitate shelter construction (Weiss et al. 2003; Yadav and Yack 2018; Henault et al. 2022), provide favorable microclimatic conditions (Tsuji and Ashford 2026), or enable rapid escape from predators (Matheson et al. 2025; Mauduit et al. 2026). Neonates may also preferentially establish in locations that minimize exposure to plant defenses, such as trichomes (Kariyat et al. 2018; Kaur et al. 2022).

Leaf trichomes play an important role in limiting caterpillar herbivory (Kaur et al. 2022). Non-glandular trichomes can physically damage caterpillars' cuticle (Gilbert 1971; Kariyat et al. 2017, 2018, 2019; Kaur and Kariyat 2020; Chowdhury et al. 2025), restrict movement, reduce attachment efficiency, and, in some cases, entrap larvae (Balakrishnan and Kariyat 2026). Glandular trichomes can chemically deter feeding by increasing exposure to sticky and/or toxic exudate that has the potential to trap or cause mortality (Malakar and Tingey 2000; Simmons et al. 2004; Lahtinen et al. 2004; Hanley et al. 2007; Ambrósio et al. 2008; Kariyat et al. 2019). Importantly, trichomes are not uniformly distributed across a leaf; their density and composition vary among regions and between adaxial and abaxial surfaces (Valkama et al. 2003; Eaton and Karban 2014; Kariyat et al. 2018; Watts and Kariyat 2021a, 2021b). This spatial heterogeneity creates a complex, leaf-scale "defensive landscape" (or "minefield") that neonates must navigate successfully to establish. Although trichome distributions can vary substantially across leaf surfaces and regions, little is known about whether neonates actively use this spatial variation when settling on an establishment site. Consequently, it remains unclear whether observed establishment patterns reflect active site selection or stochastic encounters with suitable microhabitats. Herein, we test whether neonate larvae actively select establishment sites in relation to trichome distribution using the masked birch caterpillar, *Drepana arcuata* Walker, 1855 (Drepanidae), on paper birch, *Betula papyrifera* Marshall (Betulaceae).

Drepana arcuata occurs throughout northeastern North America (Rose and Lindquist 1997; Handfield 1999) and develops through five larval instars (Yadav and Yack 2018). Early instars (I–II) are gregarious, whereas later instars (IV–V) are solitary, with the third instar representing a transitional stage. This system is particularly well-suited for studying early-instar site selection for several reasons. First, upon hatching, *D. arcuata* neonates disperse individually from the egg mass and actively search for a suitable site where they initiate feeding and construct a silk shelter (Yadav and Yack 2018). This process involves skeletonizing the leaf surface, depositing a silk mat, and building a silk shelter structure (Yadav and Yack 2018). Second, larvae may establish at different locations on a leaf (Yadav et al. 2017), suggesting that they might select among sites that differ in their defensive traits. Third, *D. arcuata* caterpillars feed primarily on species within the Betulaceae family, particularly species within the genus *Betula* spp. and *Alnus* spp. (Handfield 1999), whose leaves bear both glandular and non-glandular trichomes (Hardin and Bell 1986; Valkama et al. 2003), and in many *Betula* species, these trichomes are recognized by uneven distribution across the leaf (Valkama et al. 2003, 2005; Thitz et al. 2017; Payamnoor et al. 2019). Given the site preferences exhibited by early instar *D. arcuata* caterpillars and the potentially uneven distribution of trichomes across *Betula* leaves, we propose that trichome distribution may be a key factor in mediating site selection by *D. arcuata* neonates.

In this study, we investigate whether neonates of the masked birch caterpillar, *D. arcuata*, select specific sites to establish an initial shelter on paper birch (*B. papyrifera*) leaves in response to the distribution of non-glandular and glandular trichomes. Specifically, we: (i) quantify patterns of neonate site selection and assess whether initial placement on the leaf (i.e., on the adaxial or abaxial surface) influences the location where larvae ultimately establish; (ii) characterize the spatial distribution of glandular and non-glandular trichomes across leaf surfaces and regions; (iii) evaluate the relationship between site selection and trichome distribution; and (iv) experimentally test whether neonates avoid trichomes during feeding.

2 | Materials and Methods

2.1 | Insect and Plant

Arched hooktip moths (*D. arcuata*), adults of the masked birch caterpillar, were collected at the Queen's University Biology Station (Chaffey's Lock, ON, Canada, 44.5788° N, 76.3195° W) between May and June 2023, using a combination of mercury vapor light bulbs, ultraviolet lights, and LepiLED (Brehm 2017). Gravid females were held in glass jars (5 L) where they laid eggs on paper birch cuttings. Eggs were monitored daily, and neonates were used in experiments within the first 24 h after hatching.

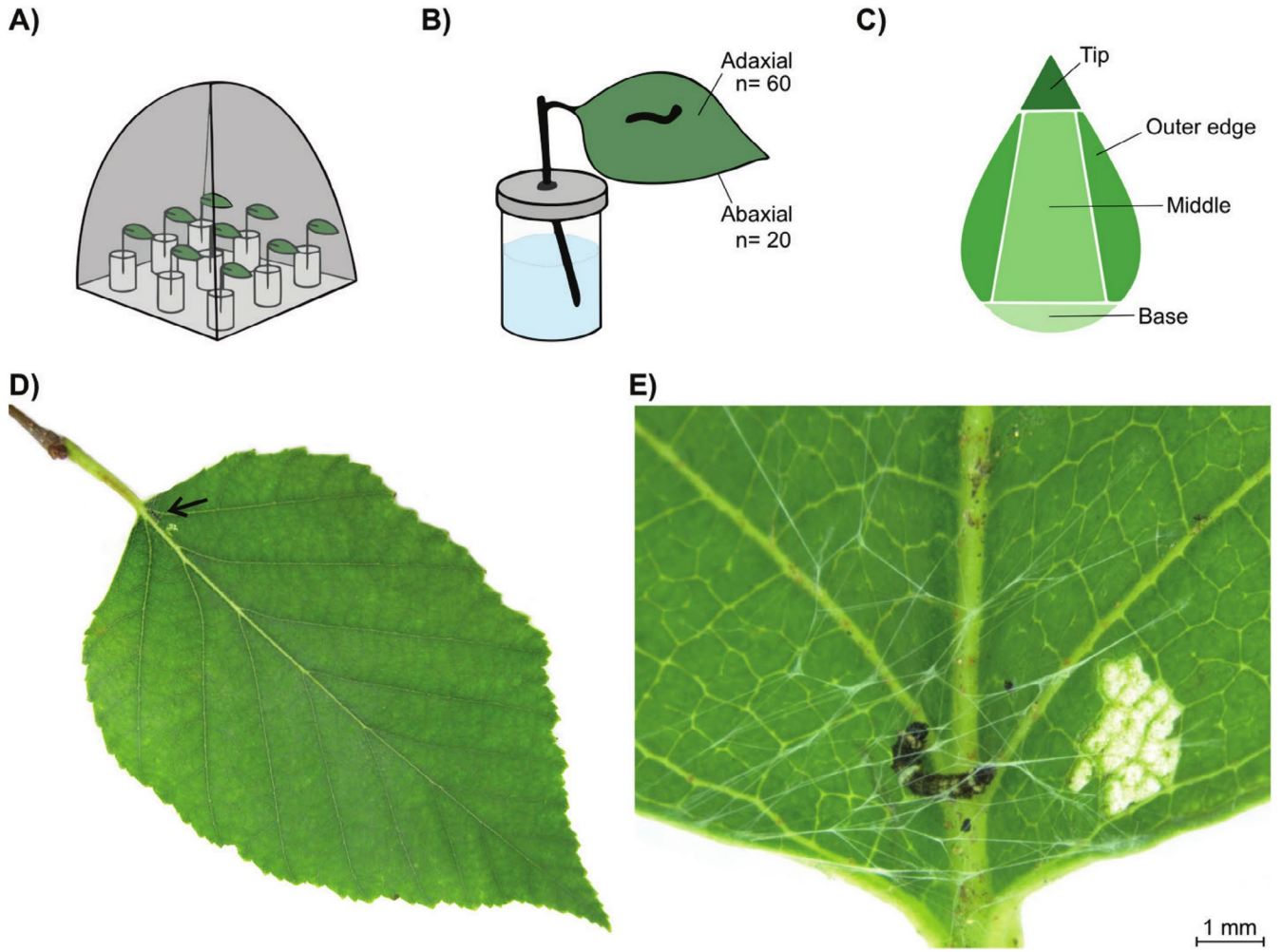

FIGURE 1 | Experimental design and criteria for larval establishment. (A) Birch cutting containing a leaf was inserted into the lid of a water-filled plastic vial. Vials were placed in mesh-covered insect enclosures, ensuring that leaves did not contact one another or the mesh. (B) On each leaf, a neonate was placed on either the adaxial ($n = 60$) or abaxial ($n = 20$) surface. (C) Spatial classification of leaf regions (base, middle, outer edge, and tip) used to record establishment sites. (D) Photograph of an entire leaf showing an establishment site (indicated by a black arrow). (E) Detail of a neonate caterpillar successfully established, defined by the presence of a feeding scar and silk deposition.

Fresh leaves of paper birch (*B. papyrifera*), a known host plant of masked birch caterpillars (Handfield 1999), were collected less than 1 h before the start of experiment from five trees at different sites in Ottawa, Ontario, Canada (Tree 1: 45.38957° N, 75.70149° W; Tree 2: 45.38367° N, 75.69961° W; Tree 3: 45.38483° N, 75.69703° W; Tree 4: 45.41311° N, 75.67568° W; Tree 5: 45.40533° N, 75.67083° W). Fully expanded leaves approximately 8–10 cm long and free of previous feeding damage were selected from each tree and used in the experiments.

2.2 | Site Selection by Neonate Caterpillars on Paper Birch Leaves

We tested whether *D. arcuata* neonates select specific sites on paper birch leaves to establish their shelter and whether initial placement of the neonate on the leaf influences subsequent choice. We used a total of 80 birch cuttings, with each containing an individual birch leaf (8–10 cm in length). Each birch cutting was inserted into the lid of a water-filled plastic vial (170 mL), and the vial was sealed using reusable adhesive putty (Staples) to prevent the larva crawling down the twig and drowning. Vials were placed in four mesh-covered insect cages (40 × 40 × 60 cm), ensuring that leaves did not contact one another or the mesh, and preventing larval escape or movement between leaves (Figure 1A). A neonate was placed at the midpoint of the leaf, with initial placement differing by leaf surface: adaxial ($n = 60$) or abaxial ($n = 20$) (Figure 1B). After an interval of 24 h, leaves were inspected to document the number of larvae established, as well as the location where the individual was established, including which side of the leaf surface (abaxial or adaxial), and the leaf region (base, middle, outer edge, or tip) (Figure 1C). A neonate caterpillar was considered "established" by the presence of a feeding scar and silk deposition (i.e., either silk mat and/or shelter) (Figure 1D,E). Subsequently, each leaf was photographed under a stereo microscope (Leica M205C; Leica Microsystems, Germany) equipped with a camera (Leica DMC4500; Leica Microsystems, Germany) to document shelter establishment and quantify trichomes at the selected sites. These data were subsequently analyzed to determine whether larvae actively avoid glandular trichomes during feeding. To assess site selection, larval preferences were first evaluated across

all individuals, independent of initial placement, for both leaf surface and leaf region. We then examined whether initial placement influenced site selection by analyzing leaf surface choice separately for larvae initially placed on (i) the adaxial surface and (ii) the abaxial surface. Leaf surface preference was tested by comparing observed frequencies with a null model assuming random choice (expected frequency = 0.5 per surface). Leaf region preference was assessed using a contingency table comparing observed frequencies with a null model of random distribution (expected frequency = 0.25 per location), irrespective of differences in zone size. Deviations from null expectation were evaluated using a chi-square test of independence implemented in the *gmodels* package (Warnes et al. 2024).

2.3 | Spatial Distribution of Trichomes on Paper Birch Leaves

To assess the distribution of trichomes on *B. papyrifera* leaves, sprigs were collected less than 1 h before the start of the experiment from the same five trees in Ottawa, Ontario, Canada (see above for coordinates). From each tree, we sampled three sprigs bearing multiple leaves at standardized vertical canopy positions: the lowest branch (low), 1 m above the lowest branch (mid), and 2 m above the lowest branch (top) (Figure 2A), resulting in a total of 15 sprigs. Four leaves per sprig were selected (60 leaves total), each measuring approximately 8–10 cm from base to tip and free of visible herbivore damage or other blemishes. Leaf surfaces were cleared of debris using an electric air pump (Coleman, Model HB-515 B). From each leaf, four circular discs (5 mm diameter; surface area = 19.63 mm^2 per side) were excised using a stainless-steel hollow punch (Walfront, Shenzhen Lanqi Technology, China) at standardized leaf region positions: base, middle, outer edge, and tip (Figure 2B), resulting in 240 samples. Each disc was placed on filter paper in a Petri dish, and both abaxial and adaxial surfaces were imaged using a Leica M205C light microscope (Figure 2C). In total, 480 images were captured, saved as TIFF files, and labeled according to tree identity (#1–5), vertical canopy position (low, mid, or top), leaf identity (#1–4), leaf region (base, middle, outer edge, tip), and leaf surface (abaxial or adaxial). All images were imported into a CorelDRAW file (Corel, Ottawa, ON) for trichome identification and quantification.

In *Betula* spp. leaves, non-glandular trichomes can occur in multiple forms (e.g., filiform, aduncate, subulate, and acicular), while glandular trichomes are represented by a single peltate type (Hardin and Bell 1986; Valkama et al. 2003, 2005; Payamnoor et al. 2019). Here, we quantified only glandular (peltate) and non-glandular (acicular) trichomes, as these are the predominant types reported for *Betula* species (Hardin and Bell 1986; Valkama et al. 2003). Glandular trichomes were identified by their circular morphology (~0.1 mm diameter) and tan-coloration with a bulls-eye pattern (Figure 2D; Figure S1A). Non-glandular trichomes were identified by their translucent to white coloration, elongated hair-like form, and a clearly visible point of insertion on the leaf surface (Figure 2E; Figure S1B). To minimize edge effects, we only included trichomes with > 50% of their structure within the sampling area. Although both glandular and non-glandular trichomes were easily recognized, their densities were measured using slightly different procedures. Glandular trichomes were counted individually and expressed as the number of trichomes per sampling area (Figure 2D). In contrast, non-glandular trichomes were sometimes too numerous and overlapping for accurate individual counts. Their density was therefore quantified using a three-level ordinal scale: "none," "low," and "high." "None" indicated a complete absence of trichomes; "low" corresponded to one or a few sparse, non-overlapping trichomes; and "high" referred to numerous trichomes that overlapped or formed clusters, making precise counts impractical (Figure 2E).

Trichome distributions were analyzed in relation to vertical canopy position (low, mid, top), leaf surface (abaxial, adaxial), and leaf region (base, middle, lateral, tip) for both glandular and non-glandular trichomes; however, as the two trichome types differed in data structure, they were analyzed using separate statistical approaches. Glandular trichome density (i.e., number of trichomes per millimeter square) data were square-root transformed and analyzed using linear mixed-effects models using the *lme4* package (Bates et al. 2015). Vertical canopy position, leaf side, and leaf location were included as fixed effects, along with their interactions. To account for the hierarchical sampling design and avoid pseudo-replication, we included tree identity, vertical canopy position within the tree, and leaf identity as nested random effects. Vertical canopy position was therefore included as a fixed effect to test its association with trichome density, while its inclusion in the random-effects structure accounted for the hierarchical sampling of leaves within trees. An initial full model including all interaction terms was fitted. Model assumptions were evaluated by inspecting residual diagnostics using the *DHARMa* package (Hartig 2024). Non-significant higher-order interactions and highly correlated terms were subsequently removed to obtain a parsimonious final model. The significance of fixed effects was assessed using Type III ANOVA, and contrasts were made using the *emmeans* package (Lenth 2025). Non-glandular trichome density, treated as an ordinal response variable (none < low < high), was analyzed using cumulative link models (CLMs) implemented in the *ordinal* package (Christensen 2025). Vertical canopy position, leaf surface, and leaf location were included as explanatory variables. Models were fitted using a logit link function. Observations were summarized by trichome category for each combination of vertical canopy position, leaf surface, and leaf location, and weighted by the number of leaves represented in each group. The significance of predictors was assessed using Type I ANOVA with Wald chi-square tests. All analyses were conducted in R software (v. 4.5.1) using the RStudio interface (R Core Team 2025; RStudio Team 2025).

2.4 | Do Neonate Caterpillars Avoid Trichomes?

To assess whether neonate *D. arcuata* avoid trichomes, we used two complementary approaches: (i) an analysis of the relationship between natural variation in trichome distribution and larval establishment patterns, and (ii) an experimental comparison of trichome density in consumed versus adjacent leaf tissue.

The first approach was to assess the relationship between trichome distribution and neonate leaf location choice. This analysis combined data from the two previously described experiments.

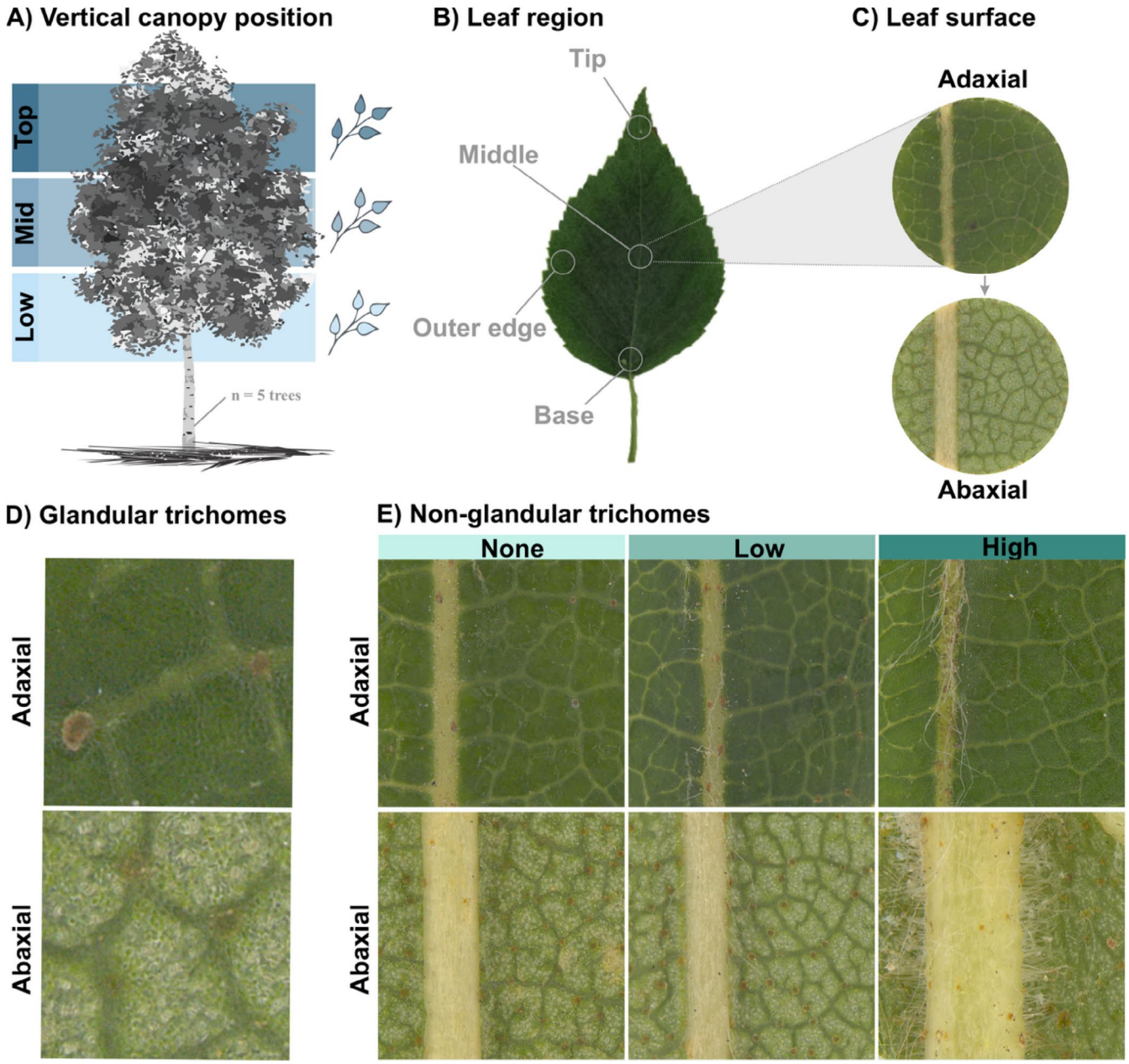

FIGURE 2 | Leaf sampling and trichome characterization on leaves. (A) Five paper birch trees were used in the experiment. From each tree, three sprigs bearing multiple leaves were sampled using tree clippers at standardized vertical positions: Lowest branch (low), 1 m above the lowest branch (mid), and 2 m above the lowest branch (top). From each sprig, four leaves were selected. (B) From each leaf, four circular discs were excised using a hollow punch at standardized positions: Base, middle, outer edge, and tip. (C) Both abaxial and adaxial surfaces of each disc were imaged using a Leica M205C light microscope. (D) Glandular trichomes were counted individually, and their density was calculated in relation to the total disc area. (E) Non-glandular trichomes were quantified using an ordinal categorical scale with three levels: "none," "low," and "high."

First, we assessed the correlation between caterpillar establishment and trichome density. For each combination of leaf surface (abaxial, adaxial) and regions (base, middle, outer edge, and tip), we quantified: (i) the number of established neonates from the site selection experiment, (ii) mean glandular trichome density, and (iii) the proportion of non-glandular trichomes within three ordinal categories (none, low, and high), with the latter two measures obtained from the spatial distribution of trichomes experiment. We then used Spearman's rank correlations to evaluate the associations between neonate establishment and these trichome variables, using *corrplot* package (Wei and Simko 2024). Second, we characterized the spatial distribution of caterpillar establishment sites in relation to trichome traits using Principal Component Analysis (PCA). Each observation corresponds to a recorded establishment site. For each establishment site, we assigned the mean glandular trichome density and non-glandular trichome category for the corresponding combination of leaf surface (adaxial or abaxial) and region (base, middle, outer edge, or tip), based on measurements from the spatial distribution of the trichome experiment. PCA was performed on standardized variables to reduce dimensionality and summarize the major axes of variation, using *FactoMineR* and *FactoExtra* package (Lê et al. 2008; Kassambara and Mundt 2020). Loadings for trichome traits were overlaid as vectors to aid interpretation

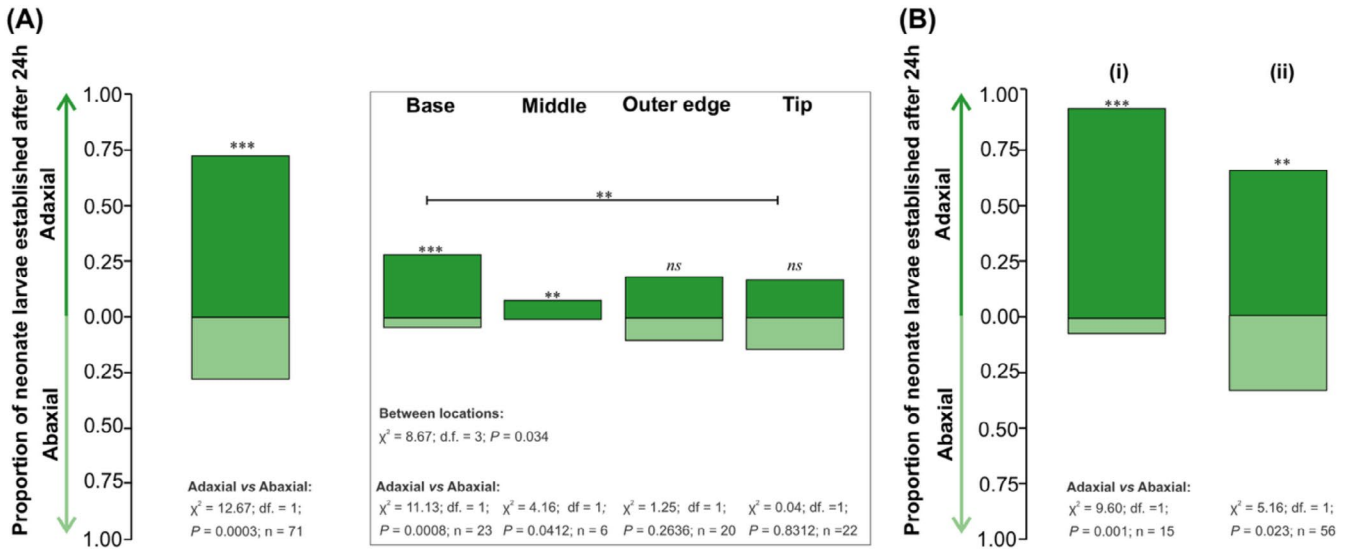

FIGURE 3 | Spatial site selection patterns by neonate caterpillars on paper birch leaves. (A) Proportion of neonates established on the adaxial (dark green) versus abaxial (light green) leaf surface and leaf regions (base, middle, outer edge, tip), independent of which surface the neonates were initially placed. Neonates showed an overall preference for the adaxial surface. Region-specific patterns indicate a significant adaxial bias at the base and middle, but not at the outer edge or tip. (B) Proportion of neonates established on abaxial versus adaxial surfaces for larvae initially placed on (i) the abaxial surface and (ii) the adaxial surface. In both scenarios, neonates preferentially established on the adaxial surface, indicating consistent surface preference independent of starting position. χ^2 statistics, degrees of freedom, *p*-values, and sample sizes (*n*) are shown below each comparison. Asterisks denote significance levels (**$p < 0.01$; ***$p < 0.001$; ns, non-significant).

of the principal components. Confidence ellipses were used to visualize clustering patterns associated with leaf surfaces and regions. To test for significant differences between leaf surfaces and regions, we performed a PERMANOVA (999 permutations) followed by a test of multivariate dispersion (betadisper) using the *vegan* package (Oksanen et al. 2025), based on Euclidean distances.

The second approach tested whether neonates actively avoid glandular trichomes during feeding. We did this by comparing images ($n = 21$ samples) of larvae established on paper birch (*B. papyrifera*) with adjacent regions of the leaf. Because feeding locations were unpredictable, pre-feeding comparisons were not feasible; therefore, we employed a paired-site approach. For each sample, we selected two adjacent areas (1.66 × 1.66 mm) within the shelter: one containing a feeding scar (consumed area) and one intact area serving as the control (non-consumed area). Within each area, we counted glandular trichomes to calculate trichome density. We then compared trichome densities between consumed and intact areas using a paired *t*-test, using the *stats* package. We assumed that similar trichome densities between areas would indicate that larvae feed around trichomes (i.e., avoidance), whereas lower trichome densities in consumed areas would suggest consumption of trichomes.

3 | Results

3.1 | Site Selection by Neonate Caterpillars on Paper Birch Leaves

In this experiment, we tested whether *D. arcuata* neonates select specific sites on paper birch leaves to establish their shelter and whether the initial placement of the neonate on the adaxial or abaxial leaf surface influences subsequent choice. Of the original 80, 71 neonates successfully established on a leaf and were thus included in our analysis. The remaining nine neonates did not construct shelters and were found wandering or dead; therefore, they were excluded from the analysis.

Neonate caterpillars exhibited a non-random site selection on paper birch leaves, showing a preference for the adaxial leaf surface and the margins of leaves. The likelihood of larvae establishing on the adaxial versus abaxial leaf surface differed significantly from random expectation ($\chi^2 = 12.67$; df = 1; $p = 0.0003$; Figure 3A), with larvae establishing more frequently on the adaxial surface over the abaxial surface. Similarly, the probability of establishing on any of the four scored leaf regions is different from that expected by chance ($\chi^2 = 8.67$; df = 3; $p = 0.034$; Figure 3A). Larvae disproportionately established at the leaf base (32.4%; $n = 23/71$), outer edge (28.2%; $n = 20/71$), and tip (31%; $n = 22/71$), whereas the middle region was used substantially less (8.4%; $n = 6/71$) (Figure 3A). Caterpillars that had established on the base of the leaf were found more frequently on the adaxial surface. All caterpillars established in the middle of the leaf were on the adaxial surface. No difference between leaf surfaces was observed for individuals establishing at the outer edge or tip (Figure 3A). Initial placement of the neonate on either the adaxial or abaxial leaf surface did not influence final surface selection. Larvae initially placed on the abaxial surface often moved to the adaxial surface ($\chi^2 = 9.60$; df = 1; $p = 0.001$; Figure 3B), whereas those initially placed on the adaxial surface generally remained there ($\chi^2 = 5.16$; df = 1; $p = 0.023$; Figure 3B). This asymmetry indicates that larvae do not passively retain their initial position, but instead actively move to preferred sites. These findings demonstrate that neonate *D. arcuata* caterpillars select specific leaf surfaces and regions,

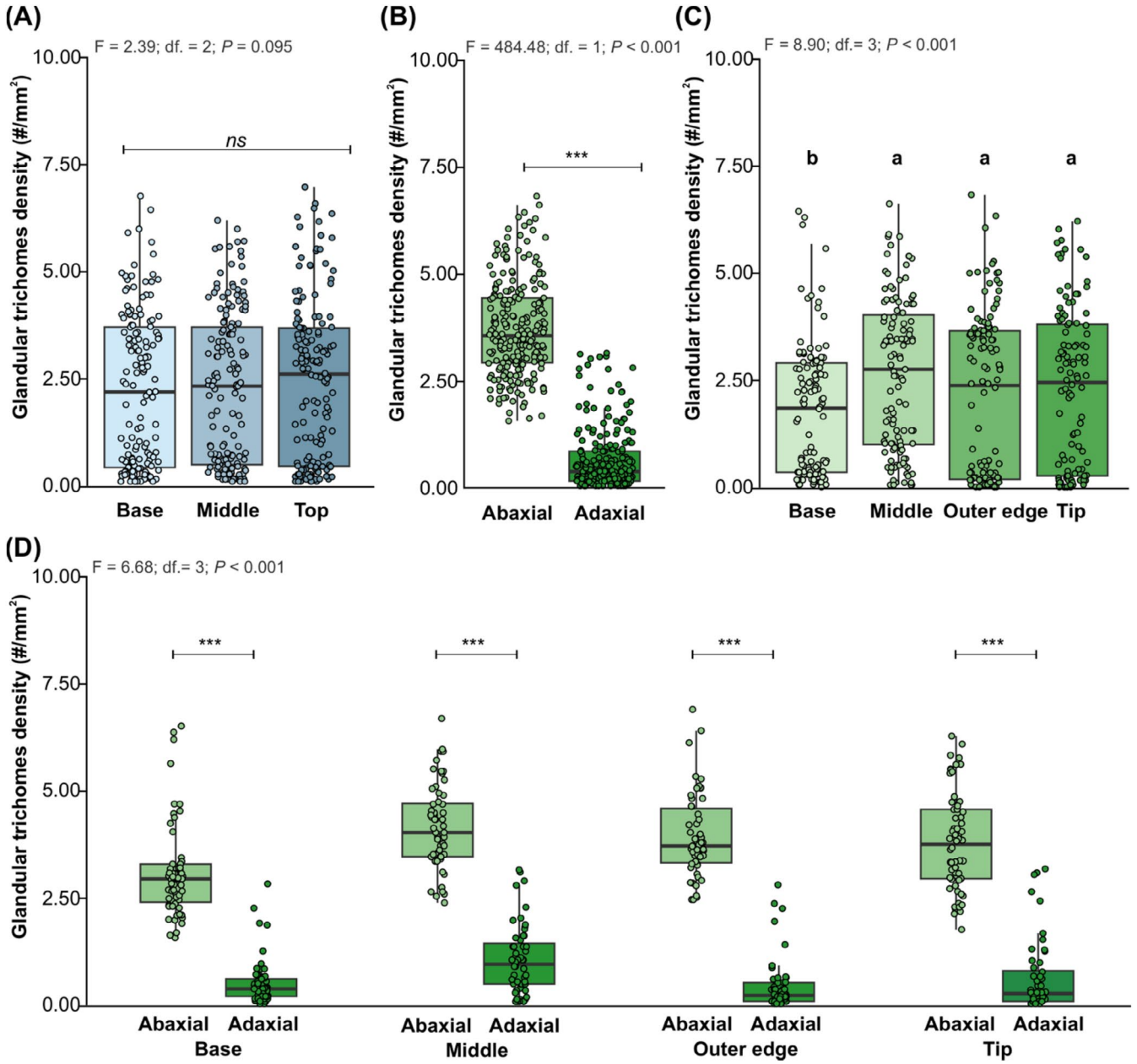

FIGURE 4 | Variation in glandular trichome density on paper birch (*Betula papyrifera*) leaves. Density of glandular trichomes is shown across (A) vertical canopy position, (B) leaf surface (abaxial vs. adaxial), (C) leaf region (base, middle, outer edge, and tip), and (D) the interaction of leaf surface and region.

favoring the adaxial surface and marginal areas on the leaf (base, outer edge, and tip) over the abaxial surface and middle regions. In addition, the observed relocation from abaxial to adaxial surfaces provides further support that site selection is an active selection process rather than a consequence of initial placement.

3.2 | Spatial Distribution of Trichomes on Paper Birch Leaves

Glandular and non-glandular trichomes were non-uniformly distributed on paper birch leaves. Trichome distribution was strongly influenced by the interaction between leaf surface and specific leaf regions.

3.2.1 | Glandular Trichomes Distribution

The glandular trichomes observed with both light microscopy and scanning electron microscopy were consistent with descriptions of peltate trichomes, with a round head measuring 70–100 μm in diameter (Hardin and Bell 1986) (Figure S1A). Glandular trichome density did not differ significantly among leaves collected from different vertical canopy positions on birch trees (low, mid, or top) ($F = 2.39$; df = 2; $p = 0.095$; Figure 4A). In contrast, trichomes were markedly more abundant on the abaxial surface than the adaxial surface ($F = 484.48$; df = 1; $p < 0.001$; Figure 4B). Trichome density also varied significantly among leaf regions ($F = 8.90$; df = 3; $p < 0.001$; Figure 4C), with the base exhibiting significantly lower density than the middle, outer edge, and tip (Figure 4C). Importantly, a significant interaction

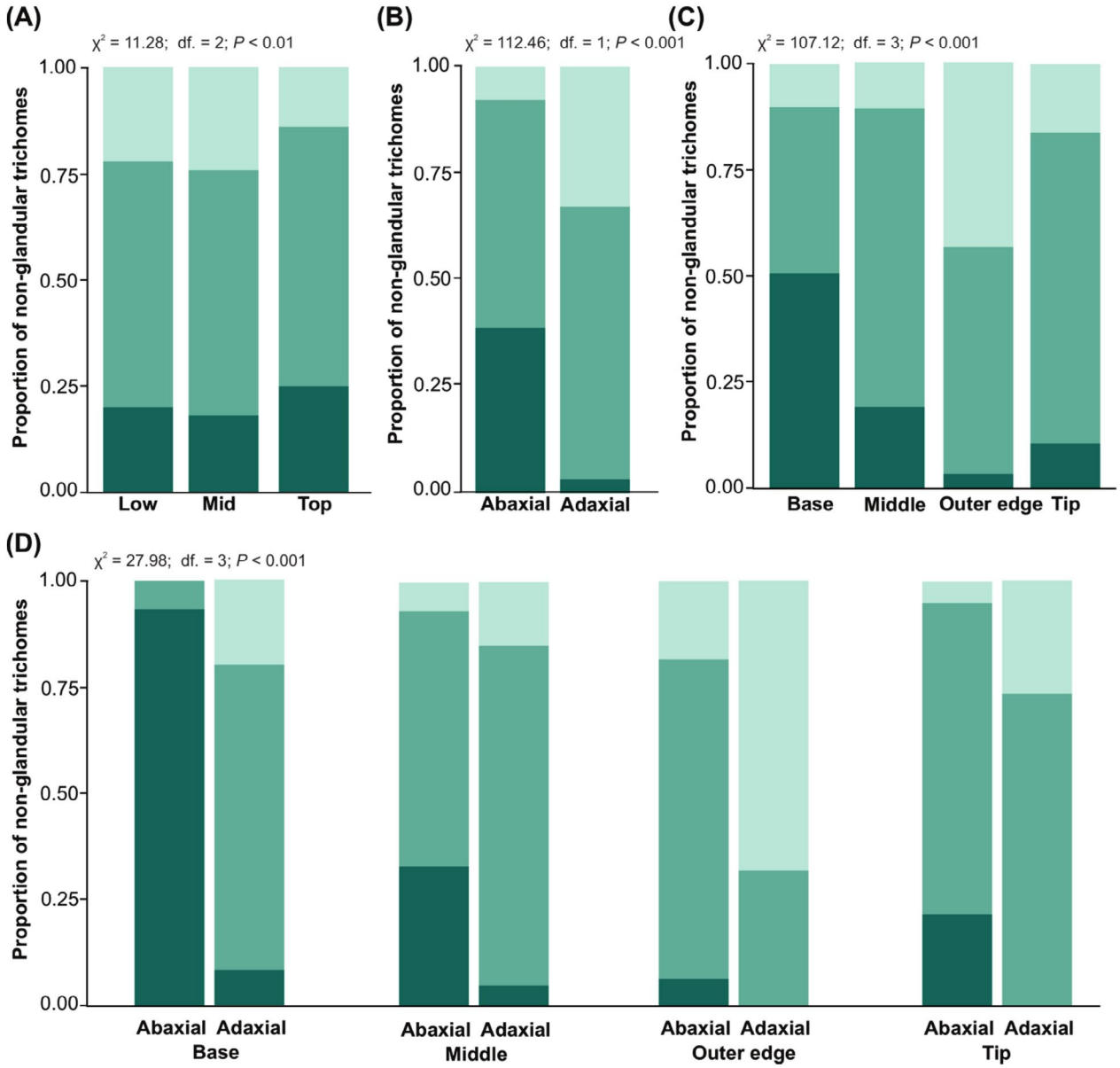

FIGURE 5 | Variation in non-glandular trichome density on paper birch (*Betula papyrifera*) leaves. Proportions of non-glandular trichomes are shown across (A) vertical canopy position, (B) leaf surface (abaxial vs. adaxial), (C) leaf region (base, middle, outer edge, and tip), and (D) the interaction of leaf surface and region. Density was quantified using a three-level ordinal scale: "none," "low," and "high." "None" (light green) indicated a complete absence of trichomes; "low" (medium green) corresponded to one or a few sparse, non-overlapping trichomes; and "high" (dark green) referred to numerous trichomes that overlapped or formed clusters, making precise counts impractical.

was observed between leaf surface and leaf region ($F = 6.68$; df = 3; $p < 0.001$; Figure 4D). The abaxial surface exhibited consistently high trichome densities across all leaf regions (> 2.50 glandular trichome/mm^2), whereas the adaxial surface exhibited low trichome densities across all leaf regions (< 0.90 glandular trichome/mm^2). This indicates that regional variation in glandular trichomes is driven primarily by patterns on the abaxial surface (Figure 4D).

3.2.2 | Non-Glandular Trichome Distribution

The non-glandular trichomes were found to be consistent with descriptions of acicular trichomes; 0.5-2 mm in length, and with "pilose" appearance (Hardin and Bell 1986). Non-glandular trichomes were recognized based on their white or translucent color, hair-like shape, and a visible origin on the leaf surface (Figure S1B). Non-glandular trichome density varied significantly according to the vertical position within the tree canopy ($\chi^2 = 11.28$; df = 2; $p < 0.01$; Figure 5A). While low- and mid-canopy leaves showed similar proportions of density categories, top-canopy leaves exhibited a shift toward a higher representation of both low- and high-density trichome categories (Figure 5A). Non-glandular trichome composition also differed strongly between leaf surfaces ($\chi^2 = 112.46$; df = 1; $p < 0.001$; Figure 5B). The abaxial surface was dominated by low- and high-density trichome categories, whereas the adaxial surface consisted primarily of low- or no-density (none) trichome categories, with high-density trichomes nearly absent. Significant variation was also observed among leaf regions ($\chi^2 = 107.12$, df = 3,

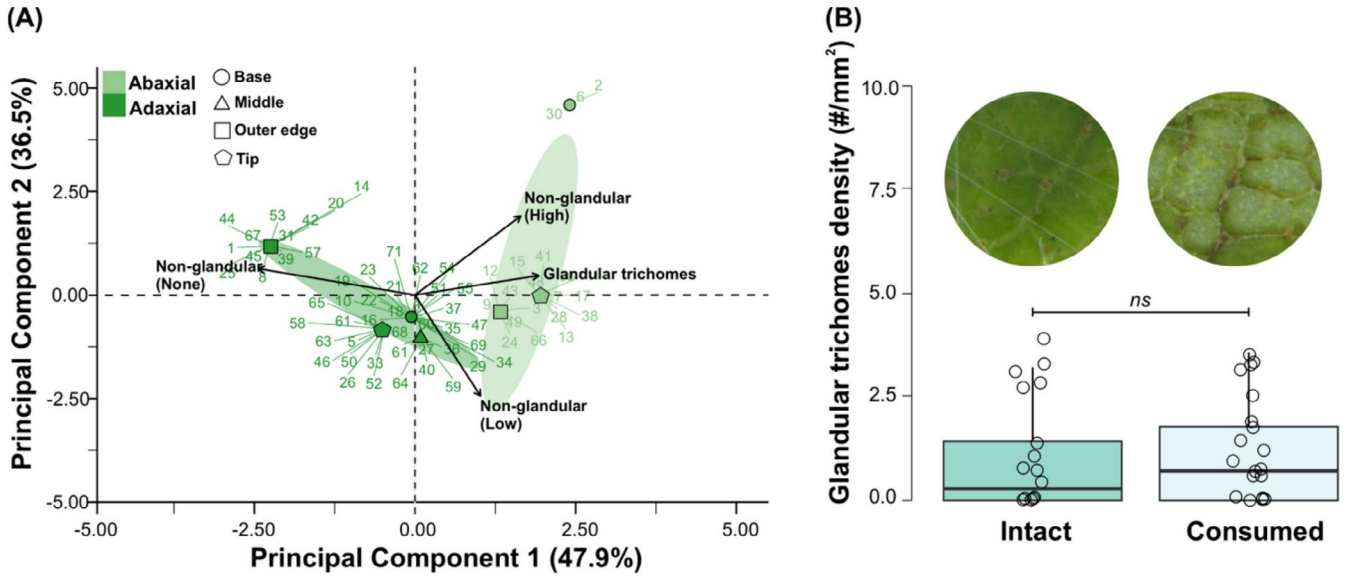

FIGURE 6 | Relationship between trichome traits and larval establishment sites. (A) Principal Component Analysis (PCA) biplot illustrating the distribution of establishment sites ($n = 71$) in relation to trichome characteristics. Each numbered point represents a sample where a neonate was established. Vectors indicate the direction and strength of trichome variables: PC1 (47.9%) separates sites by glandular trichome density, while PC2 (36.5%) reflects a gradient in non-glandular trichome coverage. Symbols represent leaf regions, and colors distinguish leaf surfaces; shaded ellipses represent 95% confidence intervals. Group differences were tested using PERMANOVA, which indicated significant clustering by leaf surface ($p < 0.001$) and leaf region ($p < 0.001$). (B) Comparison of glandular trichome density between intact and consumed (feeding scar) leaf tissue of *Betula papyrifera*. Boxplots show paired comparisons between adjacent intact and feeding (consumed) areas. Points represent individual samples. Representative light micrographs of intact (left) and consumed (right) tissue are shown above. Glandular trichome density did not significantly differ between consumed and intact areas (paired *t*-test; ns, not significant), suggesting that larvae feed selectively around glandular trichomes.

$p < 0.001$; Figure 5C). The leaf base had the highest proportion of high-density trichomes, which progressively declined toward the middle, outer edge, and tip, with increasing prevalence of low- and no-density categories, particularly at the outer edge (Figure 5C). Importantly, a significant interaction was detected between leaf surface and leaf region ($\chi^2 = 27.98$; df = 3; $p < 0.001$; Figure 5D). On the abaxial surface, high-density trichomes were predominantly observed at the base, with over 90% of samples classified in this category (Figure 5D). This proportion declined markedly toward the middle, outer edge, and tip regions, where fewer than 30% of samples exhibited high-density trichomes. In these regions, no- and low-density trichome categories were more prevalent. In contrast, on the adaxial surface, high-density trichomes were restricted to the base and middle regions, occurring in less than 10% of samples, and were completely absent from the outer edge and tip. These latter regions were instead dominated by no- and low-density trichome categories (Figure 5D).

3.3 | Do Neonate Caterpillars Avoid Trichomes?

By integrating results across experiments, we evaluated how caterpillar establishment relates to the spatial distribution of glandular and non-glandular trichomes. Overall, the patterns of site selection closely paralleled the spatial distribution of trichomes across leaf surfaces and regions. Neonate *D. arcuata* caterpillars preferentially established on the adaxial surface and at marginal regions of the leaf (base, outer edge, and tip), which correspond to areas characterized by lower trichome densities. In contrast, the abaxial surface, consistently bearing high densities of both glandular and non-glandular trichomes, was comparatively underutilized. Spearman correlation analyses supported these patterns. Establishment frequency was negatively associated with glandular trichome density ($r = -0.18$) and with both low ($r = -0.20$) and high ($r = -0.41$) densities of non-glandular trichomes. However, it shows a strong positive association with the absence of non-glandular trichomes ($r = 0.79$). These results indicate that neonates disproportionately occupy leaf areas with reduced trichome density, providing convergent evidence for trichome avoidance during initial establishment.

Principal Component Analysis further clarified these relationships. The first two principal components explained a substantial proportion of the variation in trichome traits (PC1: 47.9%; PC2: 36.5%), explaining 84.4% of the total variation (Figure 6A). PC1 primarily reflected a gradient in glandular trichome abundance, separating sites with high glandular densities (positive scores) from those with low glandular trichomes (negative scores). PC2 captured variation in non-glandular trichome density, distinguishing between areas of high and low non-glandular trichome coverage. Caterpillar establishment sites (numbered points) were broadly distributed across the ordination space but exhibited clear structure relative to these gradients. Most establishment locations clustered toward regions associated with reduced trichome density, particularly along the negative axis of PC1. In contrast, areas characterized by high glandular trichome density formed a distinct cluster that was relatively sparsely occupied, indicating avoidance rather than preference. Variation along PC2 suggests that non-glandular trichomes influence establishment in a more nuanced manner. While some caterpillars occurred in areas

with low non-glandular trichome densities, sites with very high densities were consistently avoided. The distribution of leaf positions (base, middle, outer edge, and tip; adaxial vs. abaxial surfaces) across the ordination further indicates that caterpillar establishment is shaped by the combined effects of leaf region and trichome structure. PERMANOVA also confirmed that the multivariate composition of establishment sites differed significantly between leaf surfaces ($F = 43.104$, $df = 1$; $p < 0.001$; $R^2 = 0.38$) and leaf regions ($F = 9.139$, $df = 3$; $p < 0.001$; $R^2 = 0.29$). Overall, both correlation analyses and multivariate ordination converge on the same conclusion: neonate caterpillars preferentially establish in microhabitats with reduced trichome density, supporting the hypothesis of trichome avoidance during initial establishment.

Despite this clear bias toward low-trichome microhabitats, larvae were occasionally established in regions bearing glandular trichomes. To determine whether trichomes are avoided during feeding, we compared glandular trichome densities between consumed tissue and adjacent intact areas. These comparisons revealed no significant difference in glandular trichome density (paired *t*-test: $t = 1.79$; $df = 20$; $p = 0.08$), indicating that larvae feed selectively around glandular trichomes, leaving these structures intact (Figure 6B). These findings, collectively, suggest that trichome avoidance operates across multiple scales: at a coarse scale through preferential establishment in low-trichome microhabitats, and at a finer scale through selective feeding within occupied areas.

4 | Discussion

In this study, we investigated whether neonate *D. arcuata* caterpillars establish on particular regions of birch leaves to avoid leaf trichomes. Specifically, we assessed where larvae establish on leaves, quantified the distribution of trichomes on leaves, and then evaluated whether neonates avoid leaf trichomes. Our results support the hypothesis that neonates actively avoid trichomes on leaves.

4.1 | Where Do the Neonate Caterpillars Establish?

Our results show that neonate *D. arcuata* caterpillars do not establish randomly on paper birch leaves. Rather, there were two key observations: first, they consistently established on the adaxial leaf surface, and second, they established on the marginal regions of the leaf, including the base, outer edge, and tip. We proposed that establishment site selection is an active choice rather than passive settlements for a few reasons: first, although all individuals were initially placed at the middle of the leaf, they subsequently moved to marginal regions before establishing. Second, individuals initially placed on the abaxial surface frequently relocated to the adaxial surface before establishment. In addition, previous studies have shown that females lay eggs randomly on leaves and twigs, and that neonates do not remain at their hatching sites (Yadav and Yack 2018), making a "mother knows best" explanation unlikely.

Drepana arcuata neonates showed a strong preference to establish on the adaxial surface of the leaf, and this is consistent with other drepanid species feeding on paper birch (Matheson et al. 2025; Turchen and Yack 2025) as well as a few other species of neonate caterpillars (Weiss et al. 2003; Ambrósio et al. 2008; Rodrigues et al. 2019; Henault et al. 2022). This pattern, however, contrasts with the more commonly observed behavior in neonates, which typically remain and feed on the abaxial (underside) surface of leaves, where exposure to predators, parasitoids, sunlight, and wind is often reduced (Horgan et al. 2007; Tagawa et al. 2008; Watanabe et al. 2018; Watts and Kariyat 2021a; Tsuji and Ashford 2026). While less common, establishment on the upper leaf surface may confer several advantages, including improved thermal conditions that can enhance development and metabolic activity (Wang et al. 2025), easier feeding initiation due to lower trichome density and softer leaf tissues (Hochuli 2001; Kariyat et al. 2017, 2018), reduced pathogen exposure associated with a drier microclimate, and potential behavioral benefits such as establishment of vibratory territories (Matheson et al. 2025).

Drepana arcuata neonates also showed a preference for marginal leaf regions, consistent with the observations of Yadav and Yack (2018), who report that approximately 85% of first-instar shelters were constructed along leaf margins. Similar marginal preferences have been observed in other Drepanidae species, such as *Oreta rosea* Walker 1855, and *Falcaria bilineata* (Packard 1864), although these species do not construct leaf shelters (Scott et al. 2010; Turchen and Yack 2025). Preference for leaf margins has likewise been reported in other neonate caterpillar species (Weiss et al. 2003; Greeney and Warren 2009; Henault et al. 2022). Establishment on leaf edges may offer multiple, non-mutually exclusive benefits for neonates. First, leaf edges may facilitate shelter construction through folding, rolling, or silk-mediated attachment (Weiss et al. 2003; Lill and Marquis 2007; Yadav and Yack 2018; Henault et al. 2022). Second, marginal tissues are often thinner, mechanically softer, and less structurally defended, potentially reducing the energetic and mechanical costs of initial feeding (Casher 1996). Third, positioning along leaf edges may improve escape routes during encounters with predators, parasitoids, or competitors, thereby reducing mortality risk during this vulnerable life stage (Sugiura and Yamazaki 2006; Tagawa et al. 2008; Matheson et al. 2025; Mauduit et al. 2026). Although there may be many reasons for site selection, our study specifically examined the potential role of trichomes in mediating these preferences.

4.2 | Where Are the Trichomes?

We identified two trichome types on paper birch leaves: glandular peltate trichomes and non-glandular acicular trichomes (Figure S1). These were classified based on morphological similarities with those previously described (Hardin and Bell 1986; Valkama et al. 2003, 2004). Glandular trichomes are commonly associated with chemical defense, often producing or storing sticky, deterrent, or toxic compounds that can interfere with herbivore movement and feeding (Simmons et al. 2004; Hanley et al. 2007; Farmer 2014; Kaur et al. 2022). In *Betula* spp., specifically, glandular trichomes are particularly well-suited for the secretion and retention of defensive compounds at the leaf surface, mainly flavonoid aglycones and other defensive metabolites (Hardin and Bell 1986; Valkama et al. 2003, 2005; Lahtinen

et al. 2004). In contrast, non-glandular trichomes typically function as mechanical defenses (Levin 1973; Hardin and Bell 1986; Farmer 2014; Kaur et al. 2022). Their hair-like structure imposes mechanical constraints by impeding movement, reducing attachment efficiency, and obstructing access to epidermal tissues; effects that may be especially pronounced for small-bodied neonates (Gilbert 1971; Kariyat et al. 2017, 2018, 2019; Kaur and Kariyat 2020; Chowdhury et al. 2025; Balakrishnan and Kariyat 2026).

Our results showed that both trichome types were distributed highly unevenly across the leaf in paper birch. The abaxial surface had more glandular and non-glandular trichomes (Figures 4B and 5B), a result consistent with those reported in other *Betula* species (Valkama et al. 2003, 2004; Payamnoor et al. 2019; Matsuki et al. 2021). Trichome distribution was also heterogeneous on different leaf regions, although this distribution varied depending on the type of trichome (Figures 4C and 5C). Glandular trichomes were less abundant at the base of the leaf but did not differ on the margins, whereas non-glandular trichomes showed the highest densities at the leaf base compared to the leaf margins. This within-leaf heterogeneity in trichome distribution may arise from multiple interacting factors. In *Betula* species for example, trichomes may differ substantially among genotypes (Ashton et al. 1998; Pyakurel and Wang 2013), under varying environmental conditions such as elevated CO_2 exposure, water limitation and temperature stress (Li et al. 1996; Prozherina et al. 2003; Riikonen et al. 2010; Thitz et al. 2017), across seasons (Hardin and Bell 1986; Prozherina et al. 2003), or even following prior defoliation (Rautio et al. 2002; Prozherina et al. 2003; Matsuki et al. 2021). These results suggest that birch leaves possess a multifaceted defense system combining both chemical and mechanical components. Such within-leaf heterogeneity is consistent with plant defense theory, which predicts non-uniform allocation of defenses according to tissue value, vulnerability, and developmental constraints (Levin 1973; Schoonhoven et al. 2005; War et al. 2012; Wang et al. 2021; Watts and Kariyat 2021b; Kaur et al. 2022). This fine-scale heterogeneity creates a mosaic of microhabitats, even within a single leaf; for neonate caterpillars, such variation in leaf surface defenses may mediate movement patterns, feeding-site selection, and ultimately, survival.

4.3 | Do Neonate Caterpillars Avoid Trichomes?

Our results indicate that neonates respond behaviorally to the presence of leaf trichomes when selecting microhabitats. During initial establishment, larvae consistently choose microhabitats with fewer trichomes. The positive correlation between establishment frequency and absence of non-glandular trichomes, along with negative associations with glandular and high-density non-glandular trichomes, strongly suggests that larvae actively avoid heavily defended areas during initial site selection. Principal component analysis further supports this interpretation, showing that most establishment sites clustered in regions with low trichome density. While both trichome types are associated with the avoidance of the abaxial surface during initial establishment, non-glandular trichomes appear to exert the most pronounced effect. This is particularly notable because they are rare on the adaxial surface, which is frequently selected by neonate caterpillars, whereas glandular trichomes still occur there at low densities. This pattern is consistent with previous studies showing that non-glandular trichomes are particularly effective at preventing feeding initiation by first- and second-instar caterpillars (Kariyat et al. 2018). Although glandular trichomes are often considered chemically deterrent (Lahtinen et al. 2004, 2006), dense non-glandular trichomes may impose a more immediate mechanical barrier to neonates, whose limited mobility and small body size make physical obstruction particularly costly (Kariyat et al. 2017, 2018; Kaur and Kariyat 2020; Balakrishnan and Kariyat 2026). Importantly, this avoidance behavior persisted beyond initial establishment. Larvae were not entirely excluded from tissues containing glandular trichomes; instead, they selectively fed around these structures, often leaving them intact. These results suggest that neonates continue to respond behaviorally to plant defenses after settlement and are capable of fine-scale adjustments during feeding. More broadly, our findings align with the diverse strategies herbivorous insects employ to cope with host-plant defenses (Karban and Agrawal 2002). Neonate caterpillars have been shown to avoid trichome contact (Kaur et al. 2022; Chowdhury et al. 2025), selectively feed on less-defended tissues (Connor and Taverner 1997; Sinclair and Hughes 2010; Eaton and Karban 2014; Robertson et al. 2015), and actively manipulate plant defenses (Rathcke and Poole 1975; Young and Moffett 1979; Hulley 1988; Clarke and Zalucki 2000; Musser et al. 2002; Shelomi et al. 2010; Weinhold and Baldwin 2011; Robertson et al. 2015; Dussourd 2017; Kaur and Kariyat 2020). In some systems, larvae also engage in cooperative feeding that helps overwhelm localized defenses (Fordyce and Agrawal 2001; Campbell and Stastny 2015; Despland 2019). Our results expand this framework by showing that, for *D. arcuata* neonates, a single leaf constitutes a complex defensive landscape and that newly hatched larvae exhibit sophisticated behavioral responses to cope with defensive heterogeneity at small scales.

Although our findings support a role for trichomes in mediating establishment behavior, alternative explanations should also be considered. For instance, trichomes may covary with other important leaf traits, including surface toughness, cuticle thickness, vein arrangement, epicuticular waxes, moisture content, nutritional quality, leaf age, and local microclimatic conditions, which may differ systematically between leaf surfaces and regions (Wilkens et al. 1996; Casher 1996; Eigenbrode and Pillai 1998; Kawasaki et al. 2009; Malishev and Sanson 2015; Kaur et al. 2022). Consequently, the observed behavioral pattern in neonate establishment may reflect responses to an integrated suite of structural and chemical cues rather than trichomes alone. Also, behavioral avoidance of trichomes is unlikely to be absolute, and may instead involve a trade-off. Under conditions of limited resource availability, poor host conditions, or dispersal constraints immediately after hatching, larvae may be forced to accept suboptimal sites despite the potential costs associated with trichomes. This interpretation is supported by observations that establishment can still occur in regions with high trichome density, where negative effects on neonate performance have previously been documented (Horgan et al. 2007; Kariyat et al. 2019). Thus, a caterpillar's behavioral preference for particular leaf regions may also reflect multiple, non-mutually exclusive benefits, including facilitation of shelter construction, reduced mechanical costs of feeding, and

improved escape opportunities from predators or parasitoids, as discussed previously.

5 | Conclusions

Our study demonstrates that neonate *D. arcuata* caterpillars exhibit selective establishment behavior on paper birch leaves that corresponds with the spatial distribution of leaf trichomes. Neonates preferentially occupy the adaxial surface and marginal regions, where trichome densities are lower, while avoiding heavily defended microhabitats. Selective feeding around glandular trichomes indicates that this avoidance strategy extends beyond initial establishment and continues during feeding. More broadly, our findings support the view that leaves function as complex landscapes for neonate caterpillars and such fine-scale variation can mediate neonate site-selection behavior. To further explore the proximate and ultimate explanations of microhabitat site selection, future studies might experimentally manipulate trichome density, identify the sensory mechanisms by which neonates detect and respond to localized microhabitat differences in leaf surface (England et al. 2026), and assess whether the observed site-selection behavior confers measurable fitness benefits, such as increased survival, faster development, or improved feeding efficiency.

Author Contributions

Leonardo M. Turchen: conceptualization, data curation, formal analysis, funding acquisition, investigation, methodology, project administration, software, supervision, validation, visualization, writing – original draft, writing – review and editing. **Jacob Dyck:** investigation, methodology, writing – review and editing. **Jayne E. Yack:** conceptualization, data curation, funding acquisition, methodology, project administration, resources, supervision, validation, writing – original draft, writing – review and editing.

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Ethics Statement

All applicable international, national, and institutional guidelines for the care and use of animals were considered in the present investigation.

Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available within the article and Supporting Information of this article.

References

Ambrósio, S. R., Y. Oki, V. C. G. Heleno, et al. 2008. "Constituents of Glandular Trichomes of *Tithonia diversifolia*: Relationships to Herbivory and Antifeedant Activity." *Phytochemistry* 69: 2052–2060. https://doi.org/10.1016/j.phytochem.2008.03.019.

Ang, G. C. K., R. Silva, S. L. Maxwell, M. P. Zalucki, and M. J. Furlong. 2014. "Contrary Effects of Leaf Position and Identity on Oviposition and Larval Feeding Patterns of the Diamondback Moth." *Entomologia Experimentalis et Applicata* 151: 86–96. https://doi.org/10.1111/eea.12172.

Ashton, P. M. S., L. P. Olander, G. P. Berlyn, R. Thadani, and I. R. Cameron. 1998. "Changes in Leaf Structure in Relation to Crown Position and Tree Size of *Betula papyrifera* Within Fire-Origin Stands of Interior Cedar-Hemlock." *Canadian Journal of Botany* 76: 1180–1187. https://doi.org/10.1139/b98-128.

Balakrishnan, D., and R. Kariyat. 2026. "Rice Spikelets Trap and Kill Caterpillars Using Trichomes." *Ecological Processes* 15: 26. https://doi.org/10.1186/s13717-026-00683-8.

Bates, D., M. Mächler, B. Bolker, and S. Walker. 2015. "Fitting Linear Mixed-Effects Models Using lme4." *Journal of Statistical Software* 67: 1–48. https://doi.org/10.18637/jss.v067.i01.

Brehm, G. 2017. "A New LED Lamp for the Collection of Nocturnal Lepidoptera and a Spectral Comparison of Light-Trapping Lamps." *Nota Lepidopterologica* 40: 87–108. https://doi.org/10.3897/nl.40.11887.

Campbell, S. A., and M. Stastny. 2015. "Benefits of Gregarious Feeding by Aposematic Caterpillars Depend on Group Age Structure." *Oecologia* 177: 715–721. https://doi.org/10.1007/s00442-014-3141-1.

Casher, L. E. 1996. "Leaf Toughness in *Quercus agrifolia* and Its Effects on Tissue Selection by First Instars of *Phryganidia californica* (Lepidoptera: Dioptidae) and *Bucculatrix albertiella* (Lepidoptera: Lyonetiidae)." *Annals of the Entomological Society of America* 89: 109–121. https://doi.org/10.1093/aesa/89.1.109.

Chowdhury, R., T. U. Grafe, F. Metali, and W. Federle. 2025. "Arms Race of Physical Defences: Hooked Trichomes of *Macaranga* Ant-Plants Kill Lycaenid Caterpillars, but One Specialist Has a Counter-Defence." *Biology Letters* 21: 20250005. https://doi.org/10.1098/rsbl.2025.0005.

Christensen, R. H. B. 2025. "Ordinal Regression Models for Ordinal Data." https://github.com/runehaubo/ordinal.

Clarke, A. R., and M. P. Zalucki. 2000. "Foraging and Vein-Cutting Behaviour of *Euploea core corinna* (W.S. Macleay) (Lepidoptera: Nymphalidae) Caterpillars Feeding on Latex-Bearing Leaves." *Australian Journal of Entomology* 39: 283–290. https://doi.org/10.1046/j.1440-6055.2000.00191.x.

Clissold, F. J. 2007. "The Biomechanics of Chewing and Plant Fracture: Mechanisms and Implications." In *Advances in Insect Physiology*, 317–372. Elsevier: Academic Press. https://doi.org/10.1016/S0065-2806(07)34006-X.

Cockfield, S. D., and D. L. Mahr. 1993. "Consequences of Feeding Site Selection on Growth and Survival of Young Blackheaded Fireworm (Lepidoptera: Tortricidae)." *Environmental Entomology* 22: 607–612. https://doi.org/10.1093/ee/22.3.607.

Connor, E. F., and M. P. Taverner. 1997. "The Evolution and Adaptive Significance of the Leaf-Mining Habit." *Oikos* 79: 6. https://doi.org/10.2307/3546085.

Despland, E. 2019. "Caterpillars Cooperate to Overcome Plant Glandular Trichome Defenses." *Frontiers in Ecology and Evolution* 7: 232. https://doi.org/10.3389/fevo.2019.00232.

Dussourd, D. E. 2017. "Behavioral Sabotage of Plant Defenses by Insect Folivores." *Annual Review of Entomology* 62: 15–34. https://doi.org/10.1146/annurev-ento-031616-035030.

Eaton, K. M., and R. Karban. 2014. "Effects of Trichomes on the Behavior and Distribution of *Platyprepia virginalis* Caterpillars." *Entomologia Experimentalis et Applicata* 151: 144–151. https://doi.org/10.1111/eea.12178.

Eigenbrode, S. D., and S. K. Pillai. 1998. "Neonate *Plutella xylostella* Responses to Surface Wax Components of a Resistant Cabbage (*Brassica oleracea*)." *Journal of Chemical Ecology* 24: 1611–1627. https://doi.org/10.1023/A:1020812411015.

England, S. J., C. F. McLellan, R. J. Meah, J. B. Wainwright, and L. Sumner-Rooney. 2026. "The Sensory Ecology of Caterpillars." *Journal of Comparative Physiology. A, Neuroethology, Sensory, Neural, and Behavioral Physiology* 212: 153–178. https://doi.org/10.1007/s00359-025-01778-x.

Farmer, E. E. 2014. *Leaf Defence*. Oxford University Press. https://doi.org/10.1093/acprof:oso/9780199671441.001.0001.

Fordyce, J. A., and A. A. Agrawal. 2001. "The Role of Plant Trichomes and Caterpillar Group Size on Growth and Defence of the Pipevine Swallowtail *Battus philenor*." *Journal of Animal Ecology* 70: 997–1005. https://doi.org/10.1046/j.0021-8790.2001.00568.x.

Gilbert, L. E. 1971. "Butterfly-Plant Coevolution: Has *Passiflora adenopoda* Won the Selectional Race With Heliconiine Butterflies?" *Science* 172: 585–586. https://doi.org/10.1126/science.172.3983.585.

Greeney, H. F., and A. D. Warren. 2009. "The Immature Stages and Shelter Building Behavior of *Falgo jeconia ombra* Evans, 1955 in Eastern Ecuador (Lepidoptera: Hesperiidae: Hesperiinae)." *Journal of Insect Science* 9: 1–10. https://doi.org/10.1673/031.009.3301.

Handfield, L. 1999. *Le Guide des Papillons du Québec*. Broquet.

Hanley, M. E., B. B. Lamont, M. M. Fairbanks, and C. M. Rafferty. 2007. "Plant Structural Traits and Their Role in Anti-Herbivore Defence." *Perspectives in Plant Ecology, Evolution and Systematics* 8: 157–178. https://doi.org/10.1016/j.ppees.2007.01.001.

Hardin, J. W., and J. M. Bell. 1986. "Atlas of Foliar Surface Features in Woody Plants, IX. Betulaceae of Eastern United States." *Brittonia* 38: 133–144. https://doi.org/10.2307/2807266.

Hartig, F. 2024. "*DHARMa*: Residual Diagnostics for Hierarchical (Multi-Level/Mixed) Regression Models." https://github.com/florianhartig/DHARMa.

Henault, J., D. R. Norris, J. E. Linton, and R. Westwood. 2022. "Egg Laying Behaviour and Larval Shelter-Construction Patterns of the Endangered Mottled Duskywing (*Erynnis martialis*) Butterfly's Western Population in Canada." *Journal of the Lepidopterists' Society* 76: 43–55. https://doi.org/10.18473/lepi.76i1.a6.

Hochuli, D. F. 2001. "Insect Herbivory and Ontogeny: How do Growth and Development Influence Feeding Behaviour, Morphology and Host Use?" *Austral Ecology* 26: 563–570. https://doi.org/10.1046/j.1442-9993.2001.01135.x.

Horgan, F. G., D. T. Quiring, A. Lagnaoui, and Y. Pelletier. 2007. "Variable Responses of Tuber Moth to the Leaf Trichomes of Wild Potatoes." *Entomologia Experimentalis et Applicata* 125: 1–12. https://doi.org/10.1111/j.1570-7458.2007.00590.x.

Hulley, P. E. 1988. "Caterpillar Attacks Plant Mechanical Defence by Mowing Trichomes Before Feeding." *Ecological Entomology* 13: 239–241. https://doi.org/10.1111/j.1365-2311.1988.tb00351.x.

Hurley, K. W., and D. E. Dussourd. 2015. "Toxic Geranium Trichomes Trigger Vein Cutting by Soybean Loopers, *Chrysodeixis includens* (Lepidoptera: Noctuidae)." *Arthropod-Plant Interactions* 9: 33–43. https://doi.org/10.1007/s11829-014-9348-6.

Jones, P. L., G. Petschenka, L. Flacht, and A. A. Agrawal. 2019. "Cardenolide Intake, Sequestration, and Excretion by the Monarch Butterfly Along Gradients of Plant Toxicity and Larval Ontogeny." *Journal of Chemical Ecology* 45: 264–277. https://doi.org/10.1007/s10886-019-01055-7.

Karban, R., and A. A. Agrawal. 2002. "Herbivore Offense." *Annual Review of Ecology and Systematics* 33: 641–664. https://doi.org/10.1146/annurev.ecolsys.33.010802.150443.

Kariyat, R. R., S. B. Hardison, A. B. Ryan, A. G. Stephenson, C. M. De Moraes, and M. C. Mescher. 2018. "Leaf Trichomes Affect Caterpillar Feeding in an Instar-Specific Manner." *Communicative & Integrative Biology* 11: 1–6. https://doi.org/10.1080/19420889.2018.1486653.

Kariyat, R. R., C. E. Raya, J. Chavana, J. Cantu, G. Guzman, and L. Sasidharan. 2019. "Feeding on Glandular and Non-Glandular Leaf Trichomes Negatively Affect Growth and Development in Tobacco Hornworm (*Manduca sexta*) Caterpillars." *Arthropod-Plant Interactions* 13: 321–333. https://doi.org/10.1007/s11829-019-09678-z.

Kariyat, R. R., J. D. Smith, A. G. Stephenson, C. M. De Moraes, and M. C. Mescher. 2017. "Non-Glandular Trichomes of *Solanum carolinense* Deter Feeding by *Manduca sexta* Caterpillars and Cause Damage to the Gut Peritrophic Matrix." *Proceedings of the Royal Society B: Biological Sciences* 284: 20162323. https://doi.org/10.1098/rspb.2016.2323.

Kassambara, A., and F. Mundt. 2020. "Factoextra: Extract and Visualize the Results of Multivariate Data Analyses." https://github.com/kassambara/factoextra.

Kaur, I., and R. R. Kariyat. 2020. "Eating Barbed Wire: Direct and Indirect Defensive Roles of Non-Glandular Trichomes." *Plant, Cell & Environment* 43: 2015–2018. https://doi.org/10.1111/pce.13828.

Kaur, I., S. Watts, C. Raya, J. Raya, and R. Kariyat. 2022. "Surface Warfare: Plant Structural Defenses Challenge Caterpillar Feeding." In *Caterpillars in the Middle*, edited by R. J. Marquis and S. Koptur, 65–92. Springer International Publishing. https://doi.org/10.1007/978-3-030-86688-4_3.

Kawasaki, N., T. Miyashita, and Y. Kato. 2009. "Leaf Toughness Changes the Effectiveness of Larval Aggregation in the Butterfly *Byasa alcinous bradanus* (Lepidoptera: Papilionidae)." *Entomological Science* 12: 135–140. https://doi.org/10.1111/j.1479-8298.2009.00314.x.

Lahtinen, M., L. Kapari, and J. Kenttä. 2006. "Newly Hatched Neonate Larvae Can Glycosylate: The Fate of *Betula pubescens* Bud Flavonoids in First Instar *Epirrita autumnata*." *Journal of Chemical Ecology* 32: 537–546. https://doi.org/10.1007/s10886-005-9015-6.

Lahtinen, M., J.-P. Salminen, L. Kapari, et al. 2004. "Defensive Effect of Surface Flavonoid Aglycones of *Betula pubescens* Leaves Against First Instar *Epirrita autumnata* Larvae." *Journal of Chemical Ecology* 30: 2257–2268. https://doi.org/10.1023/B:JOEC.0000048787.34388.dd.

Lê, S., J. Josse, and F. Husson. 2008. "FactoMineR: A Package for Multivariate Analysis." *Journal of Statistical Software* 25: 1–18. https://doi.org/10.18637/jss.v025.i01.

Lenth, R. V. 2025. "*emmeans*: Estimated Marginal Means, Aka Least-Squares Means." https://doi.org/10.32614/CRAN.package.emmeans.

Levin, D. A. 1973. "The Role of Trichomes in Plant Defense." *Quarterly Review of Biology* 48: 3–15.

Li, W., G. P. Berlyn, and P. M. S. Ashton. 1996. "Polyploids and Their Structural and Physiological Characteristics Relative to Water Deficit in *Betula papyrifera* (Betulaceae)." *American Journal of Botany* 83: 15–20. https://doi.org/10.1002/j.1537-2197.1996.tb13869.x.

Li, X., M. R. Berenbaum, and M. A. Schuler. 2002. "Plant Allelochemicals Differentially Regulate *Helicoverpa zea* Cytochrome P450 Genes." *Insect Molecular Biology* 11: 343–351. https://doi.org/10.1046/j.1365-2583.2002.00341.x.

Lill, J. T., and R. J. Marquis. 2007. "6 Microhabitat Manipulation: Ecosystem Engineering by Shelter-Building Insects." In *Theoretical Ecology Series*, 107–138. Elsevier. https://doi.org/10.1016/S1875-306X(07)80008-6.

Malakar, R., and W. M. Tingey. 2000. "Glandular Trichomes of *Solanum berthaultii* and Its Hybrids With Potato Deter Oviposition and

Impair Growth of Potato Tuber Moth." *Entomologia Experimentalis et Applicata* 94: 249–257. https://doi.org/10.1046/j.1570-7458.2000.00627.x.

Malishev, M., and G. D. Sanson. 2015. "Leaf Mechanics and Herbivory Defence: How Tough Tissue Along the Leaf Body Deters Growing Insect Herbivores." *Austral Ecology* 40: 300–308. https://doi.org/10.1111/aec.12214.

Marquis, R. J., and S. Koptur. 2022. *Caterpillars in the Middle: Tritrophic Interactions in a Changing World*. Springer. https://doi.org/10.1007/978-3-030-86688-4.

Matheson, S. M., L. M. Turchen, E. Mauduit, and J. E. Yack. 2025. "Buzzing Boundaries: Tiny Caterpillars Vibrate to Defend Leaf Tip Territories." *Journal of Experimental Biology* 228, no. 7: jeb.249796. https://doi.org/10.1242/jeb.249796.

Matsuki, S., R. Toki, Y. Watanabe, and K. Masaka. 2021. "Defoliation by Gypsy Moth (Lepidoptera, Erebidae) Induces Differential Delayed Induction of Trichomes in Two Birch Species." *Environmental Entomology* 50: 427–432. https://doi.org/10.1093/ee/nvaa162.

Mauduit, E., S. M. Matheson, and J. E. Yack. 2026. "Tiny Caterpillars Assess Threats by the Footsteps of Their Enemies." *Journal of Experimental Biology* 229: jeb252329.https://doi.org/10.1242/jeb.252329

Medeiros, L., and D. S. Boligon. 2007. "Adaptations of Two Specialist Herbivores to Movement on the Hairy Leaf Surface of Their Host, *Solanum guaraniticum* Hassl (Solanaceae)." *Revista Brasileira de Entomologia* 51: 210–216. https://doi.org/10.1590/S0085-56262007000200011.

Medeiros, L., and G. R. P. Moreira. 2002. "Moving on Hairy Surfaces: Modifications of *Gratiana spadicea* Larval Legs to Attach on Its Host Plant *Solanum sisymbriifolium*." *Entomologia Experimentalis et Applicata* 102: 295–305. https://doi.org/10.1046/j.1570-7458.2002.00950.x.

Musser, R. O., S. M. Hum-Musser, H. Eichenseer, et al. 2002. "Caterpillar Saliva Beats Plant Defences." *Nature* 416: 599–600. https://doi.org/10.1038/416599a.

Oksanen, J., G. L. Simpson, F. G. Blanchet, et al. 2025. "*vegan*: Community Ecology Package." https://doi.org/10.32614/CRAN.package.vegan.

Payamnoor, V., A. Sattarian, A. Kordalivand, and J. Mohammadi. 2019. "Pollen Morphology and Trichome Types of *Betula* spp. in the Hyrcanian Forests of Northern Iran." *Journal of Forestry Research* 30: 1191–1198. https://doi.org/10.1007/s11676-018-0708-4.

Prozherina, N., V. Freiwald, M. Rousi, and E. Oksanen. 2003. "Interactive Effect of Springtime Frost and Elevated Ozone on Early Growth, Foliar Injuries and Leaf Structure of Birch (*Betula pendula*)." *New Phytologist* 159: 623–636. https://doi.org/10.1046/j.1469-8137.2003.00828.x.

Pyakurel, A., and J. R. Wang. 2013. "Leaf Morphological Variation Among Paper Birch (*Betula papyrifera*; Marsh.) Genotypes Across Canada." *Open Journal of Ecology* 03: 284–295.

Qian, C., C. Wen, X. Guo, et al. 2024. "Gregariousness in Lepidopteran Larvae." *Insect Science* 31: 1353–1364. https://doi.org/10.1111/1744-7917.13312.

R Core Team. 2025. *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.r-project.org/.

Rathcke, B. J., and R. W. Poole. 1975. "Coevolutionary Race Continues: Butterfly Larval Adaptation to Plant Trichomes." *Science* 187: 175–176. https://doi.org/10.1126/science.187.4172.175.

Rautio, P., A. Markkola, J. Martel, et al. 2002. "Developmental Plasticity in Birch Leaves: Defoliation Causes a Shift From Glandular to Nonglandular Trichomes." *Oikos* 98: 437–446. https://doi.org/10.1034/j.1600-0706.2002.980308.x.

Riikonen, J., K. E. Percy, M. Kivimäenpää, et al. 2010. "Leaf Size and Surface Characteristics of *Betula papyrifera* Exposed to Elevated CO_2 and O_3." *Environmental Pollution* 158: 1029–1035. https://doi.org/10.1016/j.envpol.2009.07.034.

Robertson, G. F., M. P. Zalucki, and T. D. Paine. 2015. "Larval Host Choice of the Monarch Butterfly (*Danaus plexippus* L.) on Four Native California Desert Milkweed Species." *Journal of Insect Behavior* 28: 582–592. https://doi.org/10.1007/s10905-015-9524-2.

Rodrigues, S. M. M., N. D. C. Santos, R. M. Pitta, and J. D. N. Corassa. 2019. "Behavioral Aspects of *Helicoverpa armigera* in the Cotton Vegetative Phase." *Pesquisa Agropecuária Tropical* 49: e55628. https://doi.org/10.1590/1983-40632019v4955628.

Rose, A. H., and O. H. Lindquist. 1997. *Insects of Eastern Hardwood Trees*. Canadian Forestry Service. Service, Forestry Technical Report 29.

RStudio Team. 2025. *RStudio: Integrated Development for R*. RStudio Inc. http://www.rstudio.com/.

Schoonhoven, L. M., J. J. A. van Loon, and M. Dicke. 2005. *Insect-Plant Biology*. Oxford University Press.

Scott, J. L., S. M. Matheson, and J. E. Yack. 2010. "Variation on a Theme: Vibrational Signaling in Caterpillars of the Rose Hook-Tip Moth, *Oreta rosea*." *Journal of Insect Science* 10: 54. https://doi.org/10.1673/031.010.5401.

Shelomi, M., L. E. Perkins, B. W. Cribb, and M. P. Zalucki. 2010. "Effects of Leaf Surfaces on First-Instar *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) Behaviour." *Australian Journal of Entomology* 49: 289–295. https://doi.org/10.1111/j.1440-6055.2010.00766.x.

Simmons, A. T., G. M. Gurr, D. McGrath, P. M. Martin, and H. I. Nicol. 2004. "Entrapment of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) on Glandular Trichomes of *Lycopersicon* Species." *Australian Journal of Entomology* 43: 196–200. https://doi.org/10.1111/j.1440-6055.2004.00414.x.

Sinclair, R. J., and L. Hughes. 2010. "Leaf Miners: The Hidden Herbivores." *Austral Ecology* 35: 300–313. https://doi.org/10.1111/j.1442-9993.2009.02039.x.

Sugiura, S., and K. Yamazaki. 2006. "The Role of Silk Threads as Lifelines for Caterpillars: Pattern and Significance of Lifeline-Climbing Behaviour." *Ecological Entomology* 31: 52–57. https://doi.org/10.1111/j.0307-6946.2006.00755.x.

Tagawa, J., A. Matsushita, and T. Watanabe. 2008. "Leaf Surface Preference in the Cabbage Worm, *Pieris rapae crucivora*, and Parasitism by the Gregarious Parasitoid *Cotesia glomerata*." *Entomologia Experimentalis et Applicata* 129: 37–43. https://doi.org/10.1111/j.1570-7458.2008.00750.x.

Thitz, P., B. J. H. M. Possen, E. Oksanen, L. Mehtätalo, V. Virjamo, and E. Vapaavuori. 2017. "Production of Glandular Trichomes Responds to Water Stress and Temperature in Silver Birch (*Betula pendula*) Leaves." *Canadian Journal of Forest Research* 47: 1075–1081. https://doi.org/10.1139/cjfr-2017-0036.

Tsuji, J., and M. Ashford. 2026. "Leaf Surface Preference of *Pieris rapae* Larvae on *Brassica rapa* Under Different Light Conditions." *Bios* 97: 65–70. https://doi.org/10.1893/BIOS-D-25-00021.

Turchen, L. M., and J. E. Yack. 2025. "Warty Birch Caterpillar, *Falcaria bilineata* (Drepanidae): Ontogenetic Changes in Morphology and Behavior." *Journal of Insect Behavior* 38: 21. https://doi.org/10.1007/s10905-025-09885-y.

Valkama, E., J. Koricheva, J.-P. Salminen, et al. 2005. "Leaf Surface Traits: Overlooked Determinants of Birch Resistance to Herbivores and Foliar Micro-Fungi?" *Trees* 19: 191–197. https://doi.org/10.1007/s00468-004-0380-5.

Valkama, E., J. Salminen, J. Koricheva, and K. Pihlaja. 2003. "Comparative Analysis of Leaf Trichome Structure and Composition of

Epicuticular Flavonoids in Finnish Birch Species." *Annals of Botany* 91: 643–655. https://doi.org/10.1093/aob/mcg070.

Valkama, E., J. Salminen, J. Koricheva, and K. Pihlaja. 2004. "Changes in Leaf Trichomes and Epicuticular Flavonoids During Leaf Development in Three Birch Taxa." *Annals of Botany* 94: 233–242. https://doi.org/10.1093/aob/mch131.

Wang, H., L. Wang, X. Chen, et al. 2025. "Heat Tolerance in Tropical Moths Varies With Micro-Climate, Life Stage and Larval Feeding Strategy." *Biology Letters* 21: 20250460. https://doi.org/10.1098/rsbl.2025.0460.

Wang, X., C. Shen, P. Meng, G. Tan, and L. Lv. 2021. "Analysis and Review of Trichomes in Plants." *BMC Plant Biology* 21: 70. https://doi.org/10.1186/s12870-021-02840-x.

War, A. R., M. G. Paulraj, T. Ahmad, et al. 2012. "Mechanisms of Plant Defense Against Insect Herbivores." *Plant Signaling & Behavior* 7: 1306–1320. https://doi.org/10.4161/psb.21663.

Warnes, G. R., B. Bolker, T. Lumley, et al. 2024. "*gmodels*: Various R Programming Tools for Model Fitting." https://doi.org/10.32614/CRAN.package.gmodels.

Watanabe, T., K. Nakamura, and J. Tagawa. 2018. "Host-Plant Leaf-Surface Preferences of Young Caterpillars of Three Species of *Pieris* (Lepidoptera: Pieridae) and Its Effect on Parasitism by the Gregarious Parasitoid *Cotesia glomerata* (Hymenoptera: Braconidae)." *European Journal of Entomology* 115: 25–29. https://doi.org/10.14411/eje.2018.004.

Watts, S., and R. Kariyat. 2021a. "Picking Sides: Feeding on the Abaxial Leaf Surface Is Costly for Caterpillars." *Planta* 253: 77. https://doi.org/10.1007/s00425-021-03592-6.

Watts, S., and R. Kariyat. 2021b. "Morphological Characterization of Trichomes Shows Enormous Variation in Shape, Density and Dimensions Across the Leaves of 14 *Solanum* Species." *AoB Plants* 13: plab071. https://doi.org/10.1093/aobpla/plab071.

Wei, T., and V. Simko. 2024. "R Package '*corrplot*': Visualization of a Correlation Matrix." https://doi.org/10.32614/CRAN.package.corrplot.

Weinhold, A., and I. T. Baldwin. 2011. "Trichome-Derived *O*-Acyl Sugars Are a First Meal for Caterpillars That Tags Them for Predation." *Proceedings of the National Academy of Sciences* 108: 7855–7859. https://doi.org/10.1073/pnas.1101306108.

Weiss, M. R., E. M. Lind, M. T. Jones, J. D. Long, and J. L. Maupin. 2003. "Uniformity of Leaf Shelter Construction by Larvae of *Epargyreus clarus* (Hesperiidae), the Silver-Spotted Skipper." *Journal of Insect Behavior* 16: 465–480. https://doi.org/10.1023/A:1027399122333.

Wilkens, R. T., G. O. Shea, S. Halbreich, and N. E. Stamp. 1996. "Resource Availability and the Trichome Defenses of Tomato Plants." *Oecologia* 106: 181–191. https://doi.org/10.1007/BF00328597.

Yadav, C., R. N. C. Guedes, S. M. Matheson, T. A. Timbers, and J. E. Yack. 2017. "Invitation by Vibration: Recruitment to Feeding Shelters in Social Caterpillars." *Behavioral Ecology and Sociobiology* 71: 51–64. https://doi.org/10.1007/s00265-017-2280-x.

Yadav, C., and J. E. Yack. 2018. "Immature Stages of the Masked Birch Caterpillar, *Drepana arcuata* (Lepidoptera: Drepanidae) With Comments on Feeding and Shelter Building." *Journal of Insect Science* 18: 1–9. https://doi.org/10.1093/jisesa/iey006.

Young, A. M., and M. W. Moffett. 1979. "Studies on the Population Biology of the Tropical Butterfly *Mechanitis isthmia* in Costa Rica." *American Midland Naturalist* 101: 309–319. https://doi.org/10.2307/2424596.

Zalucki, M. P., A. R. Clarke, and S. B. Malcolm. 2002. "Ecology and Behavior of First Instar Larval Lepidoptera." *Annual Review of Entomology* 47: 361–393. https://doi.org/10.1146/annurev.ento.47.091201.145220.

Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Leaf surface micrography illustrating glandular and non-glandular trichomes in *Betula papyrifera*. (A) Glandular trichomes on adaxial (top row) and abaxial (bottom row) leaf surfaces, shown using light microscopy (left panels) and scanning electron microscopy (SEM; right panels). (B) Non-glandular trichomes on adaxial (top row) and abaxial (bottom row) leaf surfaces, shown using light microscopy (left panels) and scanning electron microscopy (SEM; right panels).