

Article

Foliar Damage Thresholds Associated with *Enallodiplosis discordis* Infestation in *Neltuma pallida* Seedlings in the Tropical Dry Forest of Northern Peru

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Abstract

Neltuma pallida is a multi-purpose tree species of the seasonally dry tropical forests of northern Peru, where it provides essential ecological and socioeconomic functions. However, recurrent defoliation associated with the cecidomyiid gall midge *Enallodiplosis discordis* may compromise early seedling establishment and the success of forest restoration programs. This study evaluated the effects of larval infestation on foliar integrity and established quantitative damage thresholds in *N. pallida* seedlings under dry forest conditions. Insects collected from naturally infested plants were identified using an integrative taxonomic approach that combined classical morphological diagnosis with COI-based DNA barcoding obtained by Sanger sequencing. Morphological assessment assigned the defoliating dipteran to *E. discordis*, while BLASTn v2.17.0. analysis of the 576-bp partial COI sequence showed 92.6% identity and 100% query coverage with Cecidomyiidae records, supporting its taxonomic placement within this family. Field bioassays conducted over a 17-week period, in which 25 individual seedlings were evaluated (N = 25), revealed a strong and significant positive correlation between larval density and foliar damage percentage ($r = 0.872$; $p < 0.001$), with moderate damage levels predominating throughout the evaluation period. Despite sustained larval presence, seedlings did not reach severe damage categories, suggesting potential relative tolerance to partial defoliation under the evaluated field conditions. Temperature and relative humidity were not significantly associated with infestation intensity or foliar damage during the study period. Overall, these findings indicate that *E. discordis*-associated foliar damage represents a relevant, although not necessarily lethal, biotic constraint for the early regeneration of *N. pallida* under the field conditions assessed. The quantitative thresholds reported here provide useful criteria for dry forest restoration programs, phytosanitary monitoring, and integrated pest management strategies in the Peruvian dry forest.

Keywords: Cecidomyiidae; defoliation; DNA barcoding; ecological restoration; foliar degradation; insect herbivory; integrated pest management; larval infestation

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1. Introduction

Seasonally dry tropical forests are among the world's most vulnerable ecosystems, increasingly threatened by climate variability, land-use change, and biotic stressors [1,2]. In northern Peru, these forests are structurally dominated by *Neltuma pallida* (Humb. & Bonpl. ex Willd.) C.E. Hughes & G.P. Lewis, formerly *Prosopis pallida*, a multi-purpose tree species that contributes to soil stabilization, nutrient cycling, microclimate regulation, and fodder provision [3,4]. Beyond its ecological role, *N. pallida* sustain rural livelihoods through food products, animal feed, beekeeping, and agroforestry uses.

Over the past decade, recurrent defoliation and the progressive decline of *N. pallida* populations have raised increasing concern regarding seedling establishment and dry forest regeneration. Although tree mortality is likely driven by multiple interacting factors, herbivorous insects are recognized as important biotic stressors in arid and semi-arid woody ecosystems [5]. Seedlings are particularly vulnerable to defoliating insects because foliar injury during early establishment can reduce photosynthetic surface area, impair carbon assimilation, and compromise subsequent growth and survival [6].

Among the phytophagous insects associated with *Neltuma* species, cecidomyiid gall midges (Diptera: Cecidomyiidae) represent an important group of herbivores capable of inducing chlorosis, necrotic lesions, foliar tissue deterioration, and premature leaflet abscission [7–10]. In the Peruvian dry forest, *Enallodiplosis discordis* has been reported as a recurrent defoliator of *N. pallida*, particularly during periods of active leaf development [7–10]. Larval activity on leaflets produces progressive surface damage associated with loss of foliar integrity, localized necrosis, and premature detachment of photosynthetic tissue. Under recurrent infestations, this damage may contribute to progressive defoliation and deterioration of foliar cover, with potential effects on plant vigor and natural dry forest regeneration [8].

Previous research has primarily contributed to the morphological characterization, taxonomic identification, symptoms of damage, and seasonal occurrence of this defoliator [9,10]. In addition, studies have also reported on its ecology, distribution, life cycle, and population behavior, assessing the level of damage using a visual rating scale—a useful methodology for describing infestation in the field [11]. Despite these observations, quantitative assessments of infestation intensity and its functional impact on foliar integrity remain limited, particularly for native tree species from seasonally dry tropical ecosystems, due to limitations in the accuracy of quantitative estimates of observed damage. Recent studies have shown that defoliation by insects can significantly reduce photosynthetic capacity, alter carbon allocation, and impair early seedling growth [12,13]. In ecological restoration contexts, identifying larval density levels associated with moderate or severe foliar damage is essential for predicting regeneration performance, defining intervention thresholds, and designing integrated phytosanitary management strategies.

In addition, woody species adapted to seasonally dry environments may exhibit tolerance mechanisms to insect-induced defoliation, including compensatory growth, physiological adjustment, and structural resilience [14]. Determining whether *N. pallida* seedlings can withstand sustained larval pressure without reaching severe damage categories provides insight into their capacity to respond to biotic stress under natural dry forest conditions.

Therefore, the main contribution of this study was to evaluate the impact of *E. discordis* infestation on foliar integrity in *N. pallida* seedlings, quantify damage thresholds under field conditions, and examine the relationship between larval density and damage severity. Direct larval counts and foliar damage estimation through random sampling enabled a more reliable quantitative assessment of defoliator infestation levels. In turn, the severity scale facilitated the standardization and comparison of damage levels observed during the evaluations, generating objective information that strengthens the understand-

ing of insect–plant interactions under field bioassay conditions. The integration of morphological and molecular identification of the insects provides a robust technical foundation and contributes valuable tools for decision-making in monitoring, management, and ecological restoration programs in the tropical dry forests of northern Peru.

2. Materials and Methods

2.1. Study Area

The study was conducted in a dry tropical forest located at the University of Piura, northern Peru ($5^{\circ}10' \text{ S}$, $80^{\circ}37' \text{ W}$), as shown in Figure 1, from August to November 2025. The area is characterized by a seasonal rainfall regime, with mean annual temperatures ranging from 20 to 24 °C and relative humidity values between 69% and 77%. This ecosystem is structurally dominated by *N. pallida*, a representative woody species of arid and semi-arid environments.

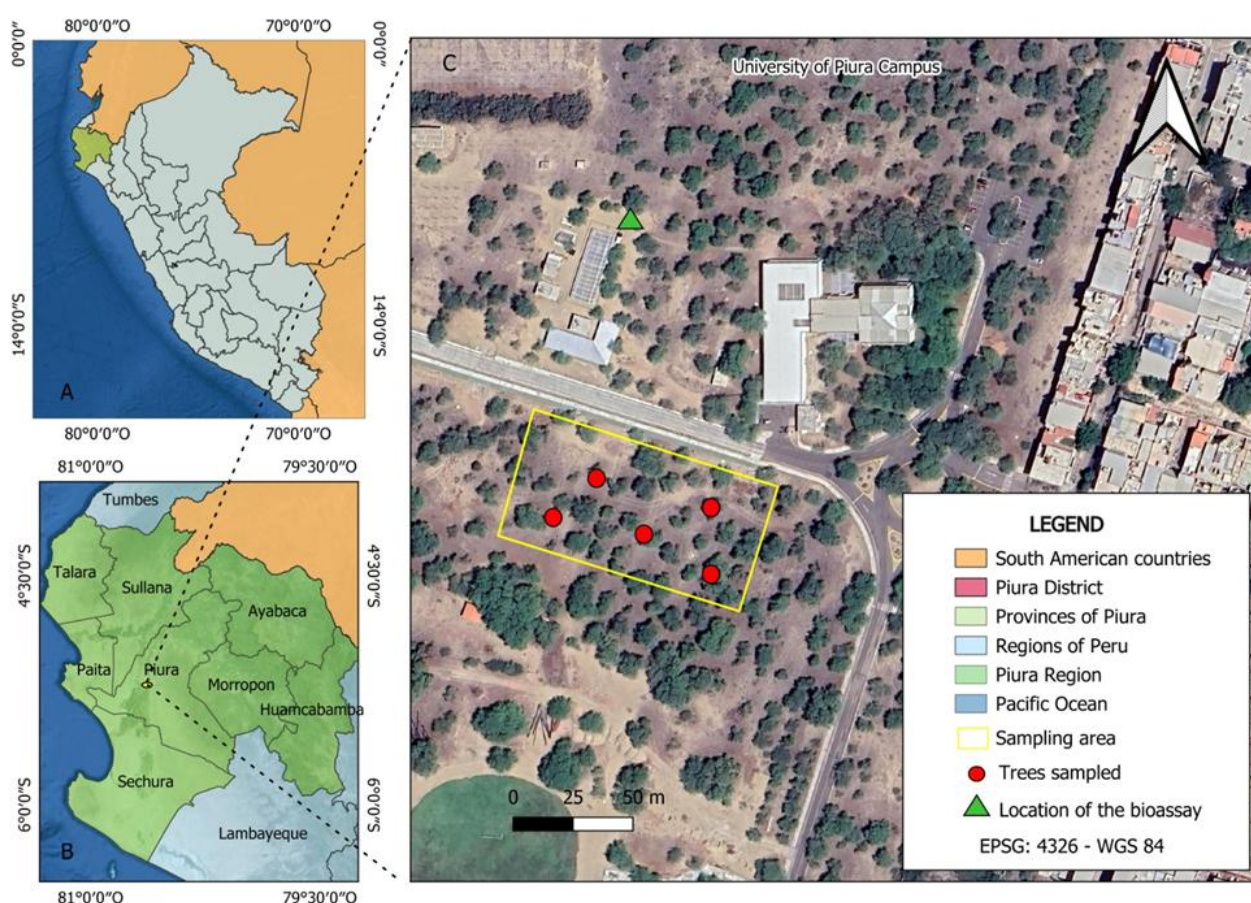


Figure 1. Geographic location of the study area and experimental site. (A) Location of northern Peru; (B) location of Piura Province, Piura; and (C) dry forest at the University of Piura. The yellow line in the satellite image delineates the sampled experimental area, whereas the green arrow indicates the location of the bioassay.

2.2. Experimental Establishment of *N. pallida* Seedlings

Mature pods were collected from healthy and vigorous *N. pallida* trees showing no visible pest damage. Seeds were manually extracted and subjected to chemical scarification with 98% sulfuric acid for 2 min to overcome physical dormancy and promote uniform germination. Germinated seeds were sown in polyethylene bags containing a homogeneous mixture of humus and fine sand at a 2:1 ratio. Two germinated seeds were placed

in each bag at a depth of 3 cm, and the most vigorous seedling was retained after emergence. Seedlings were maintained under greenhouse conditions until they reached an appropriate developmental stage for field transplantation.

Subsequently, 25 healthy, pest-free seedlings ($N = 25$) were transplanted into a 25 m² experimental plot established within the dry forest area, using a 1 m $\times$ 1 m spacing between seedlings. Each seedling was evaluated individually as an observational unit. To maintain soil surface conditions similar to those in the surrounding field environment, a thin layer of sun-dried *N. pallida* leaf litter was applied over the soil surface. Each seedling received 1 L of water weekly to prevent water stress and ensure the maintenance of functional compound leaves throughout the experimental period.

Monitoring was conducted weekly over a four-month period on *N. pallida* seedlings. At each evaluation, 15 leaves were randomly selected from each seedling, and both the number of larvae and the number of leaves exhibiting larval-induced foliar damage were recorded. These data were used to calculate the mean larval density and the mean percentage of foliar damage per week across the 25 evaluated seedlings. To minimize sampling bias, different leaves were selected at each evaluation date.

The mean percentage of foliar damage was determined as the proportion of leaves showing symptoms of yellowing and leaflet abscission relative to the total number of evaluated leaves and was expressed as a percentage. Subsequently, the mean damage values were classified according to the severity scale proposed by Mormontoy [11], adapted for application to *N. pallida* seedlings under field conditions. Each observation was assigned a corresponding damage severity level (grades 0–5) based on the established percentage intervals, as detailed in Table 1.

Table 1. Foliar damage severity scale used to evaluate *N. pallida* seedlings under field conditions.

Damage Level	Description/Damage Percentage
0	Completely healthy leaves
1	Leaves with yellowing and leaflet drop from 1% to 20%
2	Leaves with yellowing and leaflet drop from 21% to 30%
3	Leaves with yellowing and leaflet drop from 31% to 50%
4	Leaves with yellowing and leaflet drop from 51% to 75%
5	Leaves with yellowing and leaflet drop from 76% to 100%

Environmental variables were monitored over the 17-week evaluation period. Weekly mean temperature (°C) and relative humidity (%) were obtained from the meteorological station of the University of Piura and included in the analysis to assess their potential association with larval density and foliar damage intensity under field conditions.

2.3. Insect Sampling, Rearing, and Morphological Identification

Stage III larvae of a defoliating cecidomyiid associated with naturally infested *N. pallida* trees were collected from a 0.5-ha rectangular plot (50 $\times$ 100 m) located in a representative tropical dry forest of northern Peru. Within this plot, five infested trees showing damage severity level 1 were randomly selected. Samples were collected directly from the canopy, from leaves exhibiting chlorosis, leaflet abscission, and visible larval presence (Figure 2).

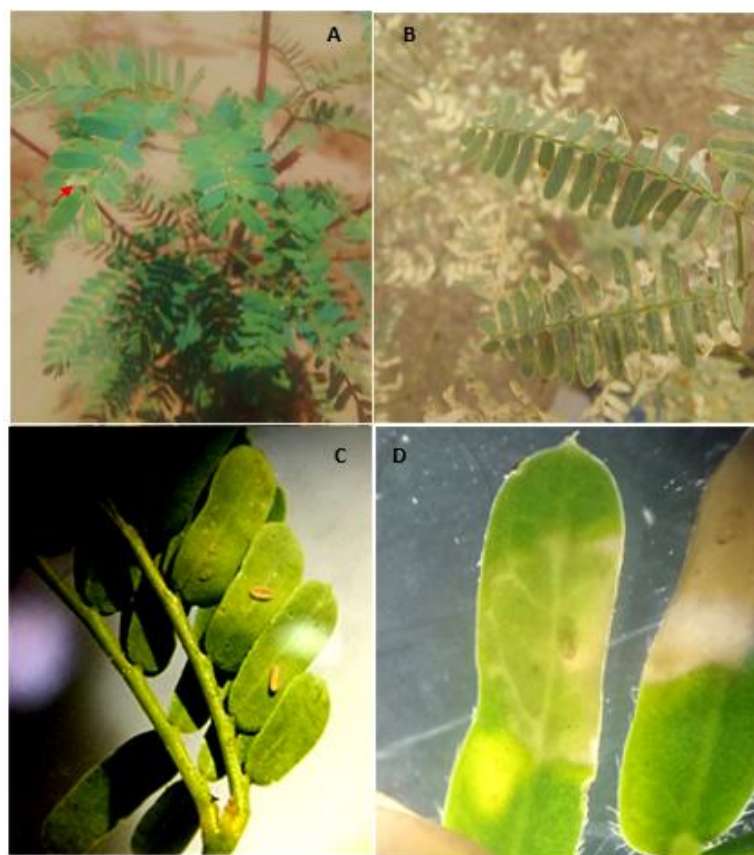


Figure 2. Characterization of foliar damage associated with *E. discordis* infestation in *N. pallida* seedlings. (A) *N. pallida* seedling infested by larvae; (B) defoliation symptoms and leaflet deterioration; (C) stage I, II, and III larvae observed under stereomicroscopy; and (D) foliar damage caused by larval activity observed under stereomicroscopy.

Larval specimens were examined under a stereomicroscope and preliminarily identified based on key morphological traits described for Cecidomyiidae larvae, including body shape, coloration, and the presence of a sternal spatula. The observed characters were compared with available taxonomic descriptions for the genus *Enallodiplosis* [7]. Representative specimens were preserved in 1.5-mL microcentrifuge tubes containing absolute ethanol for subsequent molecular analysis.

To obtain adult specimens for taxonomic confirmation, stage III larvae were transferred to rearing chambers (40 × 40 × 50 cm) containing sterilized soil (120 °C for 2 h) and sterilized organic substrate (70 °C for 1 h) to facilitate pupation. The chambers were covered with a fine mesh to prevent contamination and insect escape while allowing adequate ventilation. Emerged adults were collected and preserved in ethanol.

For classical taxonomic confirmation, adult specimens were sent to the National Phytosanitary Diagnostic Center of the National Agrarian Health Service (SENASA, Lima, Peru), where species identification was performed using diagnostic morphological criteria based on specialized taxonomic references for Cecidomyiidae.

2.4. Molecular Identification and Sequence Analysis

For molecular identification, specimens were collected from the sampling conducted for the morphological identification of the insect. This sampling step was carried out independently of the bioassay to avoid interference with the results of the assessments performed during the field bioassay.

Genomic DNA was extracted from pooled stage III larval specimens using the ZymoBIOMICS™ DNA Miniprep Kit (Zymo Research, Irvine, CA, USA), following the man-

ufacturer's instructions. DNA concentration and purity were assessed using an Implen NanoPhotometer™ NP80 spectrophotometer (Implen, Munich, Germany). Samples with A260/280 absorbance ratios between 1.8 and 2.0 were considered suitable for downstream amplification.

An approximately 600-bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using the universal primers LCO1490 and HCO2198 [15]. PCR reactions were performed using DreamTaq Master Mix (Thermo Fisher Scientific Baltics UAB, Vilnius, Lithuania) under the following cycling conditions: initial denaturation at 94 °C for 5 min; 35 cycles of denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, and extension at 72 °C for 90 s; followed by a final extension at 72 °C for 6 min.

Amplification success was verified by agarose gel electrophoresis. PCR products were purified and sequenced using Sanger sequencing technology (MR DNA, USA). Forward and reverse chromatograms were assembled, inspected, and edited in MEGA X to obtain high-quality consensus sequences. Low-quality regions at both ends of the sequences were trimmed prior to downstream analyses. Taxonomic affinity was assessed using the NCBI BLAST+ suite v2.17.0. algorithm against sequences deposited in the GenBank database of the National Center for Biotechnology Information (NCBI). The availability of COI reference sequences for *E. discordis* in GenBank was also verified during the database search. The generated COI sequence was submitted to NCBI/GenBank for evaluation and publication as a reference barcode for *E. discordis*, designated as isolate PERU01. Additional information is provided in Supplementary Material File S1.

2.5. Statistical Analysis

Descriptive statistics were calculated for larval density, foliar damage percentage, and damage severity to summarize central tendency and dispersion throughout the evaluation period. For each variable, the mean, standard deviation, minimum, and maximum values were estimated.

Pearson correlation analysis was performed to assess the relationships among larval density, foliar damage percentage, damage severity, mean temperature, and relative humidity. Correlation coefficients (r) were interpreted according to the strength and direction of the associations between biological and environmental variables.

All statistical analyses were performed using SPSS v.27.0 (IBM Corp., Armonk, NY, USA). Statistical significance was set at $p < 0.05$.

3. Results

3.1. Morphological Confirmation of *E. discordis* and Molecular Placement Within Cecidomyiidae

Three larval stages associated with foliar damage in *N. pallida* were morphologically distinguished. Stage I larvae were translucent to creamy yellow, with an ellipsoidal and segmented body measuring 0.21–0.30 mm in length. Stage II larvae were pale yellow to orange, with visible dark-brown setae and lengths ranging from 0.70 to 0.82 mm. Stage III larvae were dark yellow to orange, showed slight dorsoventral flattening, and measured 1.1–2.4 mm in length. These traits were consistent with available morphological descriptions of Cecidomyiidae larvae and, together with the taxonomic assessment of adults, supported the classical identification of the defoliating insect as *E. discordis* [7].

Amplification of the mitochondrial cytochrome c oxidase subunit I (COI) gene generated single, well-defined PCR products in pool of larvae, indicating specific and reproducible amplification. After Sanger sequencing and chromatogram editing, a COI consensus sequence was obtained and used for taxonomic comparison through BLASTn. The analysis showed 92.59% identity with a mitochondrial COI sequence assigned to *Cecidomyiidae* sp. in NCBI/GenBank (*Cecidomyiidae* sp. voucher BIOUG1117-F04, COI gene; ac-

cession number: KT108297.1), supporting the molecular affinity of the defoliating insect within this family. However, because this level of identity does not provide robust molecular confirmation at the species level and, at the time of analysis, no previously described COI sequences for *E. discordis* were available in GenBank, the species assignment was based primarily on classical morphological characterization. Consequently, the COI sequence generated in this study was submitted to NCBI/GenBank for evaluation and publication as a molecular reference associated with specimens morphologically identified as *E. discordis*.

3.2. Foliar Damage Characterization

Infestation by *E. discordis* produced visible foliar lesions, initially characterized by pale circular halos at larval attachment sites. Progressive surface feeding resulted in chlorotic areas that subsequently developed into necrotic patches as damage advanced. Sustained feeding activity caused desiccation of the affected leaflets followed by their abscission, indicating a pattern of progressive foliar degradation associated with larval presence (Figure 2).

3.3. Temporal Dynamics of Larval Density and Foliar Damage

No larvae were recorded during the first two weeks of evaluation. From week 3 onward, larval density increased progressively, reaching a population peak between weeks 5 and 7, with a maximum of 87 larvae recorded across the experimental plot. A secondary increase was observed during weeks 15 and 16. The results revealed marked temporal variation in larval population density, characterized by successive phases of absence, a gradual increase, a population peak, and a subsequent decline accompanied by secondary increases, suggesting an unstable insect–seedling population dynamic. Foliar damage followed a similar temporal pattern, increasing in parallel with larval abundance during the main infestation peak and exceeding 20% at the time of maximum population density. As larval density declined, foliar damage levels also decreased, suggesting a temporary reduction in active larval pressure on the foliage (Figure 3). This temporal correspondence indicates a direct positive relationship between fluctuations in larval density and the intensity of foliar damage. Overall, damage severity remained within the slight to moderate categories throughout the study period and did not reach severe damage levels.

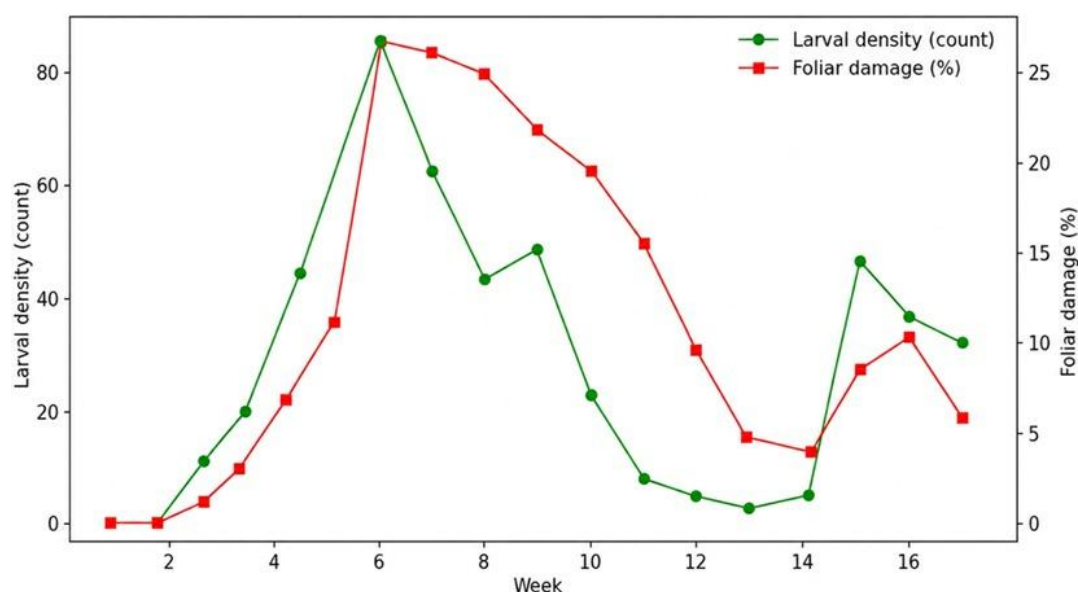


Figure 3. Temporal dynamics of larval density and foliar damage in *N. pallida* seedlings during the evaluation period from August to November 2025.

3.4. Descriptive Statistics

Descriptive statistics for the 17 weekly evaluations are presented in Table 2. Mean larval density was 29.47 ± 25.20 individuals, whereas mean foliar damage reached $14.65 \pm 8.66\%$. The average damage severity score was 1.24 ± 0.66 , indicating a predominance of mild to moderate damage throughout the evaluation period. These values, together with the high standard deviation in larval density, reinforce the existence of pronounced temporal variability in the pest population, consistent with a fluctuating rather than a stable population pattern. The environmental variables, presented in Table 2, exhibited limited variability, with minimum and maximum temperatures of 20.03°C and 23.74°C , respectively, and minimum and maximum relative humidity values of 69.34% and 77.41% , respectively, indicating relatively stable field conditions throughout the bioassay period. This environmental stability suggests that the observed fluctuations in larval density were more likely driven by the biological dynamics of the insect and its interaction with the seedlings than by pronounced changes in climatic conditions.

Table 2. Descriptive statistics of larval density, foliar damage, damage severity, and environmental variables recorded during 17 weekly evaluations.

Variable	Minimum	Maximum	Mean	Standard Deviation
Number of larvae	0	87	29.47	25.20
Foliar damage (%)	0	26.87	14.65	8.66
Damage severity score	0	2	1.24	0.66
Temperature ($^\circ\text{C}$)	20.03	23.74	21.79	0.87
Relative humidity (%)	69.34	77.41	73.44	2.16

3.5. Relationship Between Environmental Variables, Larval Density, and Foliar Damage

A strong and significant positive relationship was observed between larval density and foliar damage percentage ($r = 0.872$; $p < 0.001$). Simple linear regression further confirmed that larval density explained a substantial proportion of the variation in foliar damage ($R^2 = 0.757$; $p < 0.001$), indicating that increasing larval abundance was associated with greater foliar injury in *N. pallida* seedlings (Figure 4).

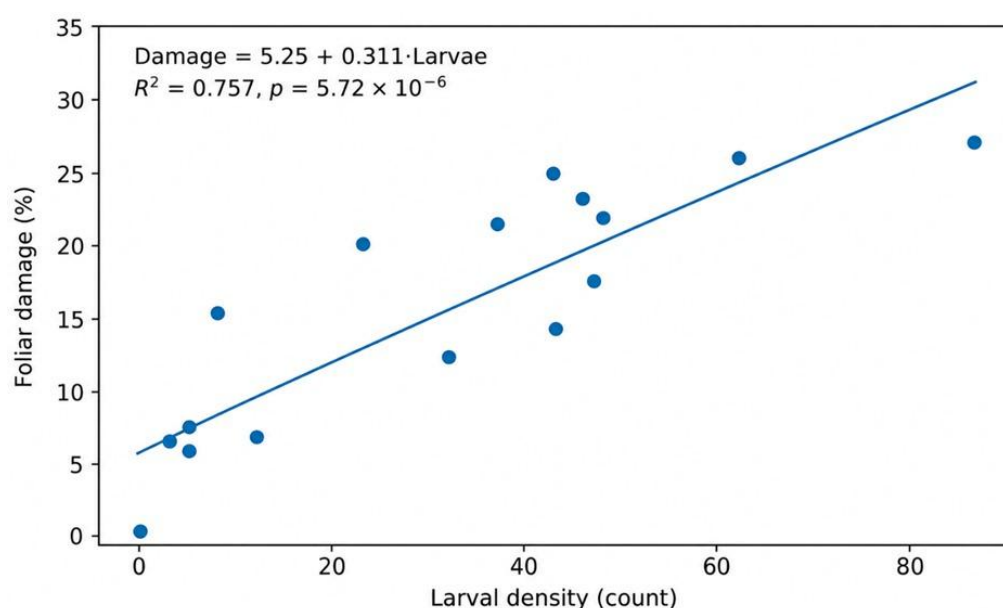


Figure 4. Linear regression between larval density and foliar damage percentage in *N. pallida* seedlings.

3.6. Environmental Variables and Correlation Analysis

Temperature and relative humidity remained relatively stable throughout the evaluation period, with mean values of 21.79 ± 0.87 °C and $73.44 \pm 2.16\%$, respectively (Figure 5). Pearson correlation analysis showed no significant associations between temperature and larval density ($r = -0.080$), foliar damage percentage ($r = -0.083$), or damage severity score ($r = -0.039$). Similarly, relative humidity was not significantly correlated with larval density ($r = -0.386$), foliar damage percentage ($r = -0.455$), or damage severity score ($r = -0.381$). In contrast, a significant negative correlation was observed between temperature and relative humidity ($r = -0.703$; $p = 0.002$), reflecting the expected inverse relationship between these environmental variables under the evaluated field conditions (Table 3).

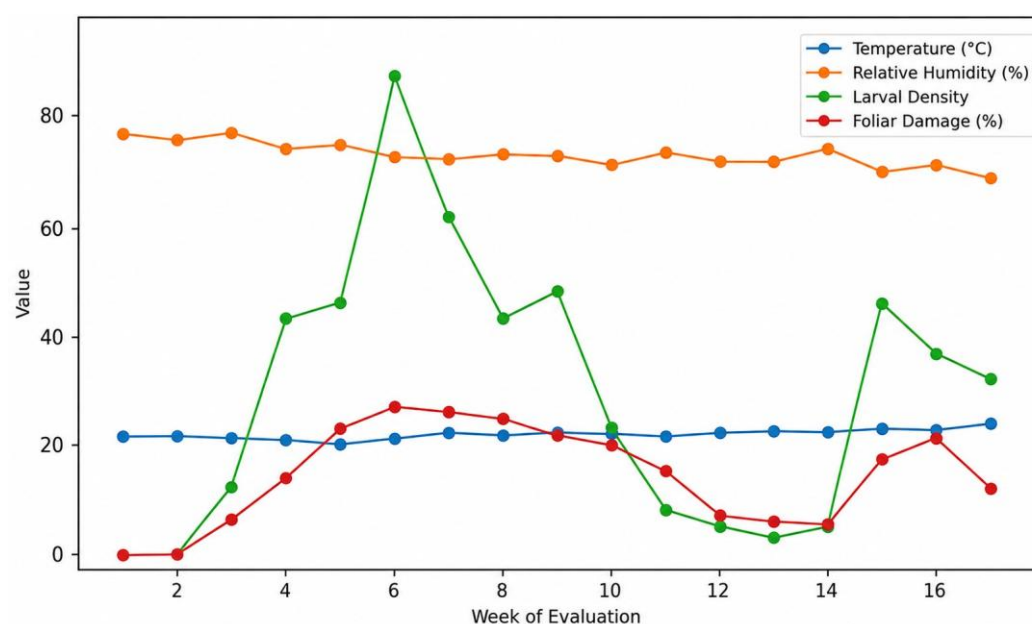


Figure 5. Temporal variation in temperature, relative humidity, larval density, and foliar damage percentage in *N. pallida* seedlings during the 17-week field evaluation.

Table 3. Pearson correlation coefficients among larval density, foliar damage percentage, damage severity score, temperature, and relative humidity recorded during the 17-week field evaluation.

Variable	Number of Larvae	Foliar Damage (%)	Damage Severity Score	Temperature	Relative Humidity
Number of larvae	1	0.872 *	0.766 *	−0.080	−0.386
Foliar damage (%)	0.872 *	1	0.874 *	−0.083	−0.455
Damage severity score	0.766 *	0.874 *	1	−0.039	−0.381
Temperature	−0.080	−0.083	−0.039	1	−0.703 *
Relative humidity	−0.386	−0.455	−0.381	−0.703 *	1

* Significant correlation at $p < 0.05$.

4. Discussion

The progressive decline of *N. pallida* represents a direct threat to the persistence and functional stability of the tropical dry forest of northern Peru, given the structural, ecological, and socioeconomic role of this species within these ecosystems. The recent taxonomic recircumscription separating *Neltuma* from *Prosopis* sensu stricto further highlights the need to use updated nomenclature and accurate taxonomic traceability in ecological, phytosanitary, and restoration studies involving the species commonly known as “algarrobo” [4]. In this context, increasing defoliation and dieback events reported along the northern

Peruvian coast have been linked to the activity of phytophagous insects, including *E. discordis*, a cecidomyiid recognized as a relevant defoliator of *Prosopis/Neltuma* in Peru and Chile [8].

4.1. Molecular Identification and Reference Gaps in Cecidomyiidae

In the present study, mitochondrial COI sequence analysis allowed the evaluated specimens to be molecularly placed within the family Cecidomyiidae. BLASTn comparison showed 100% query coverage and 92.6% nucleotide identity with available COI records in GenBank assigned to Cecidomyiidae. This result indicates that the obtained sequence fully aligned with the homologous region available in the database, although the identity value was below that typically expected for robust species-level confirmation in taxonomic groups with well-represented reference libraries.

This limitation does not invalidate the taxonomic assignment based on morphological characters; rather, it reflects the absence of previously recorded COI sequences for *E. discordis* in GenBank and the limited molecular representation of closely related lineages within Cecidomyiidae. Consequently, the molecular result should be interpreted as complementary evidence of affinity at the family level, while the specific identification of the specimens as *E. discordis* was based primarily on morphological characterization, along with the correspondence between the observed diagnostic characters, the host, and the pattern of leaf damage described for this species. This interpretation is consistent with the symptoms recorded in *N. pallida*, including superficial scraping of leaf tissue, lesions associated with larval attachment sites, and the progressive defoliation reported for *Neltuma* [8]. Likewise, the absence of specific molecular references coincides with the limitations described for hyperdiverse and taxonomically complex groups, where a high proportion of Neotropical lineages lack molecular assignment at the species level [16]. Therefore, the COI sequence generated in this study constitutes a first step toward bridging this reference gap and strengthening future molecular comparisons of *E. discordis* and other Cecidomyiidae associated with *N. pallida*.

4.2. Relationship Between Larval Density, Foliar Integrity, and Operational Threshold Definition

Field bioassays with *N. pallida* seedlings showed that increasing larval density was consistently associated with higher foliar damage, as demonstrated by the strong positive correlation between these variables and the linear model, which explained a large proportion of the observed variation in damage percentage (Figure 4). This pattern is biologically consistent with the feeding behavior described for *E. discordis*, whose larvae remain attached to the leaflets and progressively expand superficial scraping lesions, promoting chlorosis, localized necrosis, leaflet abscission, and loss of functional photosynthetic area [8,9].

From a restoration perspective, the main contribution of these findings is not merely the confirmation of foliar damage, but the translation of larval pressure into operational categories of mild, moderate, and severe injury. This approach links larval density to quantifiable levels of foliar integrity loss, enabling early identification of conditions that may compromise seedling performance under field conditions. Recent evidence indicates that herbivory can exert particularly relevant effects during early plant developmental stages, due to limited reserve availability, reduced compensatory capacity, and high dependence on active photosynthetic surface area; however, the magnitude of these effects may vary according to species identity, ontogenetic stage, and environmental context [17]. Thus, defining thresholds based on the relationship among larval density, damage percentage, and severity score provides an applied tool for dry forest restoration programs.

4.3. Potential Adaptive Response and Compensation in *N. pallida*

The predominance of mild to moderate damage, without progression to severe categories during the evaluation period, suggests that *N. pallida* seedlings may exhibit some degree of functional tolerance to partial defoliation under the assessed environmental conditions. Plant tolerance to herbivory, including compensatory growth responses, depends on species-specific functional traits, the ontogenetic stage, resource availability, and the intensity and duration of herbivore pressure [18]. In woody species adapted to arid and semi-arid environments, physiological plasticity, resprouting capacity, and adjustment of growth patterns may help buffer the effects of moderate foliar loss. In *N. pallida*, its ecological plasticity and adaptation to the climatic variability typical of seasonally dry tropical forests may partly explain the absence of widespread severe damage observed in this study [19].

The absence of severe damage categories suggests that the larval densities of *E. discordis* recorded during the evaluation period did not reach the levels required to cause critical loss of leaf tissue in *N. pallida* seedlings. Furthermore, the tolerance of this species to herbivory, along with its potential for leaf compensation, may have helped limit the progression of damage to more severe categories. These results indicate that, under the evaluated conditions, the impact of the infestation remained primarily within the range of light to moderate damage.

This interpretation is consistent with recent evidence showing high morphological variability among *N. pallida* populations, including differences in height, stem diameter, and fruit production associated with environmental heterogeneity, soil fertility, and organic matter content [20]. These findings suggest that the species has the capacity to adjust growth and performance according to local conditions. However, because the present study primarily evaluated foliar integrity over a limited period, potential physiological or morphological compensation in response to insect-induced defoliation should be interpreted as a plausible ecological hypothesis rather than as a demonstrated mechanism.

Insect–plant interactions may change under more stressful climatic conditions, particularly when high temperature and water limitation occur simultaneously. Warming can increase insect population growth, feeding activity, reproduction, and range expansion, whereas water stress may alter host plant quality and susceptibility to insect-induced foliar damage [21]. However, empirical responses are context dependent, with insect damage reported to increase or decrease in water-stressed plants depending on the insect feeding guild, host traits, and environmental conditions [22,23]. In this study, the bioassay was conducted from August to November under relatively stable conditions and with weekly irrigation, which may have reduced seedling water stress. Therefore, the predominance of mild to moderate foliar damage should be interpreted within this seasonal context. Under summer conditions, higher temperatures, greater evaporative demand, and reduced water availability could modify both *E. discordis* performance and *N. pallida* susceptibility, potentially changing the severity and frequency of damage. Future evaluations across contrasting climatic periods are needed to refine the interaction among insect pressure, plant response, and environmental stress.

4.4. Environmental Variables

The environmental variables recorded during the evaluation period showed relatively low variability and were not significantly correlated with larval density or foliar damage. This suggests that, under the assessed conditions, infestation dynamics were more closely associated with biological interactions between the insect and its host plant than with short-term climatic fluctuations. Similar observations have been reported in studies on *Prosopis/Neltuma* decline in northern Peru, where tree health dynamics result from complex interactions among biotic agents, environmental stress, and ecosystem-

level processes rather than from isolated climatic variables [24]. Consequently, monitoring programs focused exclusively on environmental thresholds may overlook early stages of herbivore outbreaks, highlighting the importance of directly assessing infestation levels and foliar damage in field management programs.

4.5. Implications for Restoration and Integrated Management

From a restoration perspective, understanding the relationship between herbivore pressure and plant performance is essential to improve the establishment success of native species in degraded seasonally dry tropical forest ecosystems. The observed relationship between larval density and foliar damage provides quantitative evidence that may contribute to defining operational damage thresholds during early plant development. Integrating these thresholds into monitoring protocols could help anticipate potential effects on seedling vigor and survival, thereby supporting adaptive management strategies in restoration initiatives. Seasonally dry tropical forest restoration frequently faces multiple constraints, including herbivory, water stress, and anthropogenic disturbance. Incorporating ecological indicators, such as herbivore-induced foliar damage levels, may improve decision-making and help reduce early seedling mortality in restoration programs [25].

Overall, the findings of this study highlight the ecological relevance of *E. discordis* as a defoliating agent associated with foliar damage in *N. pallida* seedlings while also suggesting that this tree species may retain a degree of resilience under moderate infestation levels. Continuous monitoring of insect–plant interactions in tropical dry forest ecosystems will help refine operational damage thresholds and improve understanding of the herbivory dynamics affecting the regeneration and conservation of *N. pallida* populations.

5. Conclusions

The present study provides new evidence on the interaction between the defoliating insect *E. discordis* and *N. pallida* seedlings under conditions of the tropical dry forest of northern Peru. Taxonomic identification of *E. discordis* was supported by morphological traits, larval feeding biology, and the observed pattern of foliar damage. Molecular analysis based on the mitochondrial COI gene supported the taxonomic placement of the analyzed specimens within the family Cecidomyiidae; however, species-level molecular assignment was limited by the absence of COI reference sequences for *E. discordis* and the limited representation of this group in public databases such as GenBank.

Field bioassays showed that larval density was strongly and positively associated with foliar damage in *N. pallida* seedlings. Under the evaluated conditions, increasing infestation intensity resulted in higher levels of foliar injury, indicating that larval density represents a useful indicator of insect-induced damage to the foliar integrity of this species.

Despite sustained larval presence, the observed damage remained predominantly within mild to moderate levels, suggesting a degree of tolerance of *N. pallida* seedlings to partial defoliation during the evaluation period. This response may be related to the ecological plasticity of the species and its adaptation to the variable conditions characteristic of tropical dry forest ecosystems. However, this interpretation should be considered within the temporal context of the study, which was conducted during a specific season and under relatively stable environmental conditions.

Temperature and relative humidity were not significantly associated with larval density or foliar damage during the evaluated period. This suggests that, under these conditions, infestation dynamics were mainly associated with the biological interaction between the insect and its host plant rather than with short-term climatic variation. Nevertheless, insect–plant responses may differ under contrasting climatic scenarios, particularly during periods of higher temperature, water deficit, or increased physiological stress

in seedlings, which may alter both insect development and feeding behavior, as well as plant susceptibility to foliar damage.

Overall, these results contribute to a better understanding of the effect of *E. discordis* on foliar integrity in *N. pallida* seedlings and provide a quantitative basis for assessing insect-induced foliar damage in dry forest restoration and monitoring programs. Incorporating indicators such as larval density and foliar damage into monitoring protocols may facilitate the early detection of infestation events and support adaptive management strategies for the conservation and recovery of algarrobo populations. However, the management implications derived from the identified larval thresholds should be interpreted with caution, given that their operational effectiveness was not evaluated through experimental interventions in this study. Consequently, further research is needed to validate these reference values under field conditions through the implementation of management actions, including insecticide treatments or other control strategies applied at different infestation levels relative to the established thresholds. This approach will help determine the predictive capacity and practical utility of these thresholds as decision-making tools in ecological restoration programs and adaptive management of tropical dry forests. Future studies should extend evaluations across contrasting climatic seasons and water-stress conditions to refine understanding of the interactions among *E. discordis*, *N. pallida*, and the environment. Likewise, it is essential to explicitly evaluate the interaction between herbivory and drought stress, as these factors may exert combined effects on the physiological performance of the host plant, the population dynamics of the herbivore, and the severity of the associated damage. Investigating these processes will provide a more robust ecological framework for predicting the response of tropical dry forests to climate change scenarios and will contribute to the development of conservation and management strategies supported by scientific evidence.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/ijpb17070053/s1>, File S1 (Partial COI sequence of *Enallodiplosis discordis* isolate PERU01).

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