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# Ontogenetic variation in susceptibility of Spodoptera frugiperda (Lepidoptera: Noctuidae) to azadirachtin under high-altitude conditions: an integrated dose–response and survival analysis approach

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# Abstract

Azadirachtin, the principal bioactive limonoid of Azadirachta indica A. Juss., is a widely used botanical insecticide in Integrated Pest Management programs targeting Spodoptera frugiperda (J.E. Smith), yet the ontogenetic modulation of its toxicity under high-altitude Andean thermal conditions remains uncharacterized. This study quantified the susceptibility of six larval instars of S. frugiperda to azadirachtin under high-mountain laboratory conditions (2,586 m a.s.l.; 17 ± 1°C) using an integrated framework combining Probit dose–response analysis, Kaplan–Meier survival estimation, and Cox proportional hazards regression. Survival analysis was performed on 1,488 time-to-event records (n = 248 per instar; 1,009 events, 479 right-censored observations). Early instars (L1–L3) exhibited high susceptibility, with 87.9–89.9% cumulative mortality within 96 h, whereas late instars (L4–L6) showed markedly lower mortality (42.7–51.6%), with more than half of the individuals surviving the observation period. Kaplan–Meier curves differed significantly among instars (log-rank combined trend: $\chi^2 = 119.35$, df = 1, $p < 0.001$). The Cox proportional hazards model was highly significant ($\chi^2 = 95.77$, df = 2, $p < 0.001$): each successive larval instar reduced instantaneous mortality hazard by approximately 15.4% (HR = 0.846; 95% CI: 0.816–0.878), while each 100 µL/L increment in azadirachtin concentration increased mortality risk by 2.6% (HR = 1.026; 95% CI: 1.013–1.039). The convergence between dose–response and survival modeling confirms a consistent ontogenetic gradient in susceptibility under cooler Andean thermal conditions and provides a quantitative framework for timing neem-based interventions in high-altitude maize agroecosystems.


# 1 INTRODUCTION

*Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae), the fall armyworm, represents one of the most economically significant constraints on maize production in Neotropical and, increasingly, global agroecosystems. Since its detection in sub-Saharan Africa in 2016, the species has expanded across multiple continents, threatening food security on an unprecedented scale.[1] The rapid global spread of *S. frugiperda* has been accompanied by the emergence and spread of resistance to synthetic insecticides — including organophosphates, pyrethroids, spinosyns, and Bt toxins — in field populations from Brazil, Mexico, and Asia.[2] These resistance developments have intensified interest in botanical insecticides as sustainable alternatives within Integrated Pest Management (IPM) frameworks, particularly in regions where small-scale farmers lack access to expensive chemical inputs.

Azadirachtin, the principal bioactive limonoid extracted from seeds of *Azadirachta indica* A. Juss. (Meliaceae), represents one of the most thoroughly characterized botanical insecticides in the entomological literature. Its multi-modal mechanism of action — encompassing antifeedant activity, disruption of ecdysone biosynthesis, inhibition of molting-regulatory nuclear receptors, and impairment of reproductive fitness — makes resistance development significantly less likely than for single-target synthetic compounds.[3,4] Critically, azadirachtin's primary toxicological effects are most pronounced during active growth and molting phases, suggesting that larval developmental stage may be a major determinant of field efficacy.

Despite an extensive literature on azadirachtin toxicity against lepidopteran pests, two important knowledge gaps persist. First, virtually all published bioassay data for *S. frugiperda* have been generated under lowland tropical laboratory conditions (20–30°C), which do not represent the Andean highland agroecosystems where substantial maize production occurs at elevations of 1,800–3,000 m a.s.l. under mean temperatures of 14–18°C. Temperature is known to profoundly alter both toxicokinetic (absorption, distribution, metabolism) and toxicodynamic (receptor sensitivity, target enzyme activity) processes in insects,[5] and published dose recommendations derived from lowland bioassays may therefore be inappropriate for highland applications. Second, conventional toxicological endpoints — particularly the median lethal concentration ($LC_{50}$) at a fixed time point — provide limited information about the temporal dynamics of insecticide-induced mortality. This matters because field applications of botanical insecticides often operate within constrained exposure windows and knowing whether a compound kills quickly or slowly is essential for predicting real-world efficacy.

Survival analysis methods, including the Kaplan–Meier estimator and the Cox proportional hazards regression model, offer a fundamentally different — and complementary — framework for characterizing insecticide toxicity. Rather than asking 'what fraction of organisms die at a given dose?', these methods ask 'how quickly do organisms die, and how does the hazard of death change over time as a function of covariates?'.[6] This time-to-event approach explicitly accounts for right-censored observations (individuals that survive to the end of the observation period), which conventional ANOVA and $LC_{50}$ analyses must discard or treat as arbitrary. The Cox model additionally provides hazard ratios (HR) that quantify the proportional change in instantaneous mortality risk associated with each unit change in a covariate — a parameter directly interpretable in applied decision-making contexts.

In a companion study using Probit dose–response analysis and lethal time estimation (Giraldo-Vanegas et al., in press), we characterized $LC_{50}$ and $LT_{50}$ values for all six larval instars of *S. frugiperda* exposed to azadirachtin under the same high-altitude conditions, identifying a critical physiological threshold at the L3–L4 transition. The present study extends that analysis by applying survival analytical methods — Kaplan–Meier estimation and Cox regression — to the same experimental dataset, providing a time-to-event perspective that complements and strengthens the Probit-based findings. The specific objectives were to: (i) estimate and compare Kaplan–Meier survival functions across larval instars and azadirachtin concentrations; (ii) test the significance of differences among survival curves using log-rank tests; (iii) quantify the independent effects of larval instar and concentration on instantaneous mortality hazard using Cox proportional hazards regression; and (iv) assess the consistency between dose–response and survival-based toxicological frameworks for characterizing ontogenetic susceptibility gradients.

# 2 MATERIALS AND METHODS

## 2.1 Insect colony, bioassay conditions, and experimental design

The bioassay system has been described in detail in the companion Probit analysis paper (Giraldo-Vanegas et al., in press); only the elements essential for interpreting the survival analysis are summarized here. Larvae of *Spodoptera frugigiperda* were reared under controlled conditions at 2,586 m a.s.l. (17 ± 1°C, 65 ± 10% RH, 12:12 h L:D photoperiod) on a semi-artificial maize-based diet. All six larval instars (L1–L6) were exposed individually to seven azadirachtin concentrations (0, 275, 550, 833, 1,108, 1,383, and 1,667 µL/L of commercial neem oil formulation; 1.2% EC equivalent to 12 g azadirachtin/L) via a leaf-dip method. Treated maize leaf sections were offered to individual larvae confined in Petri dishes; mortality was recorded at 12, 24, 36, 48, 60, 72, 84, and 96 h post-exposure. The experiment comprised 1,050 experimental units (7 concentrations × 5 replicates × 5 larvae × 6 instars), with 25 larvae per concentration per instar.

# 2.2 Survival data structure and coding

For survival analysis, the experimental data were restructured into a time-to-event format. Each individual larva constituted one record, characterized by: (i) the time of death in hours (event time), or (ii) the end of the observation period (96 h) for larvae that survived throughout (right-censored observations). The event indicator was coded as 1 (death observed) or 0 (censored/survived). Larvae in the absolute control group that survived to 96 h were coded as censored. The total analytical dataset comprised 1,488 records across all six instars (n = 248 per instar): 1,009 events (67.8%) and 479 right-censored observations (32.2%). Censoring rates differed markedly by instar: L1–L3 had 10.1–12.1% censored observations, whereas L4–L6 had 48.4–57.3%, reflecting their substantially lower susceptibility within the 96 h observation window.

# 2.3 Kaplan–Meier survival estimation

Non-parametric survival functions were estimated separately by larval instar (pooled over concentrations) and by azadirachtin concentration (pooled over instars) using the Kaplan–Meier product-limit estimator.[7] Differences among survival curves were assessed by the log-rank (Mantel–Cox) test,[8] applied both pairwise and as a combined trend test using an ordered weight vector (− 5, − 3, −1, 1, 3, 5) for the six concentration groups. A combined trend chi-square statistic ($\chi^2 = 119.35$, df = 1) was computed to evaluate the overall monotonic relationship between concentration and survival time. All analyses were performed in IBM SPSS Statistics v.26.0 (IBM Corp., Armonk, NY, USA).

# 2.4 Cox proportional hazards regression

A Cox proportional hazards regression model[9] was fitted to quantify the simultaneous and independent effects of larval instar (ordinal predictor, levels 1–6) and azadirachtin concentration (continuous predictor, scaled per 100 µL/L increment) on the instantaneous mortality hazard. The proportional hazards assumption was assessed graphically by inspection of log-minus-log survival plots across covariate strata and by evaluating time-dependent covariate interactions; no meaningful deviations were observed within the experimental time frame. Model fit was evaluated using the global likelihood ratio test statistic ($\chi^2$). Hazard ratios ($HR = \exp(\beta)$) and their 95% confidence intervals were reported. Adjusted

survival curves were generated at the mean of covariates (instar = 3.5; concentration = 938.1 µL/L) as implemented in SPSS.

# 3 RESULTS

## 3.1 Descriptive survival patterns: events and censoring by instar

The overall event rate across all instars was 67.8% (1,009 deaths out of 1,488 larvae within 96 h). However, this aggregate figure conceals a pronounced ontogenetic gradient in the probability of dying within the observation window (Table 1). Instars L1, L2, and L3 showed consistently high event rates of 87.9%, 89.1%, and 89.9%, respectively, indicating that nearly nine in ten larvae from these instars died within 96 h of exposure. In sharp contrast, instars L4, L5, and L6 showed event rates of only 51.6%, 42.7%, and 45.6%, respectively, with the majority of larvae in L5 surviving to the end of the observation period. The step-change in event rate between L3 (89.9% mortality) and L4 (51.6% mortality) — a 38.3 percentage-point reduction within a single instar transition — constitutes the most striking single finding of this analysis and identifies the L3–L4 boundary as a qualitative shift in azadirachtin susceptibility under high-mountain thermal conditions.

Table 1
Survival analysis summary by larval instar of Spodoptera frugiperda exposed to azadirachtin under high-mountain conditions (17 ± 1°C; 2,586 m a.s.l.) over 96 h.

| Instar | n total | Events (deaths) | Censored (survived) | Cumulative mortality at 96 h (%) | Terminal survival probability S(96 h) |
|---|---|---|---|---|---|
| L1 | 248 | 218 | 30 | 87.9 | 0.121 |
| L2 | 248 | 221 | 27 | 89.1 | 0.109 |
| L3 | 248 | 223 | 25 | 89.9 | 0.101 |
| L4 | 248 | 128 | 120 | 51.6 | 0.484 |
| L5 | 248 | 106 | 142 | 42.7 | 0.573 |
| L6 | 248 | 113 | 135 | 45.6 | 0.544 |

*Note: Events = observed deaths within 96 h. Censored = individuals surviving to end of observation period (right-censored). Terminal survival probability S(96 h) estimated by Kaplan–Meier product-limit method.*

## 3.2 Kaplan–Meier survival curves by larval instar

Kaplan–Meier survival curves stratified by larval instar (all concentrations pooled) showed clear visual and statistical separation among developmental stages (Fig. 1). Instars L1, L2, and L3 produced nearly superimposable survival curves with steep initial declines beginning at 12 h post-exposure and reaching terminal survival probabilities of 0.121, 0.109, and 0.101, respectively, at 96 h. Instars L4, L5, and L6

maintained substantially higher survival probabilities throughout the observation period, with terminal values of 0.484, 0.573, and 0.544, respectively. The median survival time (time at which S(t) = 0.50) was reached within the observation window for L1, L2, L3, and L4, whereas the median survival time for L5 and L6 was not reached within 96 h, indicating that the majority of individuals in these instars survived the experiment at the tested concentrations. Log-rank tests confirmed highly significant differences in survival distributions among all instar pairs ($p < 0.001$).

## 3.3 Kaplan–Meier survival curves by azadirachtin concentration

Survival curves stratified by concentration (all instars pooled) demonstrated a consistent dose-dependent effect (Fig. 2). The control group (0 µL/L) maintained high survival throughout the 96 h period, with a terminal survival probability close to 1.0; differences among instars within the control were not significant (log-rank $\chi^2 = 5.00$, df = 5, $p = 0.416$), confirming bioassay validity. All treated concentrations (275–1,667 µL/L) produced statistically significant reductions in survival relative to controls ($p < 0.001$ for all). The most pronounced separation from the control was observed at 550 µL/L (log-rank $\chi^2 = 53.47$, $p < 0.001$), followed by 833 µL/L ($\chi^2 = 34.28$, $p < 0.001$) and 1,667 µL/L ($\chi^2 = 21.72$, $p = 0.001$). The combined trend test across the ordered concentration gradient confirmed a highly significant monotonic relationship between concentration and survival time ($\chi^2 = 119.35$, df = 1, $p < 0.001$), consistent with a dose-dependent toxicological effect.

Instar-stratified survival curves by concentration (Fig. 3) revealed that the dose-dependent separation of survival curves was greatest for early instars (L1–L3), where concentrations as low as 275 µL/L produced steep, rapid survival declines. For late instars (L4–L6), the curves showed markedly less separation across concentrations and flatter, delayed mortality trajectories, indicating both reduced overall susceptibility and reduced concentration sensitivity in older larvae. Notably, at 550 µL/L — the concentration with the highest overall log-rank statistic — early instar survival fell to zero by 84–96 h, whereas L5 and L6 survival remained above 0.20–0.25 even at the highest tested concentration (1,667 µL/L), suggesting that a ceiling effect of azadirachtin's lethal action exists for late instars within the tested time frame.

dependent toxicological effect.

## 3.4 Cox proportional hazards model

The Cox proportional hazards model incorporating larval instar and azadirachtin concentration was globally significant ($\chi^2 = 95.77$, df = 2, $p < 0.001$), confirming that both covariates independently and significantly modulate the instantaneous mortality hazard (Table 2). Model parameters and hazard ratios are presented in Table 2.

Table 2
Cox proportional hazards regression model evaluating the effects of larval instar and azadirachtin concentration on mortality risk of Spodoptera frugiperda under high-mountain conditions (17 ± 1°C; 2,586 m a.s.l.).

| Predictor | β | SE | Wald $\chi^2$ | p | HR = exp(β) | 95% CI (HR) |
|---|---|---|---|---|---|---|
| Larval instar (ordinal, L1–L6) | −0.167 | 0.019 | 77.78 | < 0.001 | 0.846 | 0.816–0.878 |
| Concentration (per 100 µL/L) | 0.025 | 0.006 | 15.89 | < 0.001 | 1.026 | 1.013–1.039 |

*Note: Global model: $\chi^2 = 95.77$, df = 2, $p < 0.001$. N = 1,488 (1,009 events, 479 censored). Covariate means: instar = 3.50; concentration = 938.1 µL/L. HR = hazard ratio; CI = confidence interval. The proportional hazards assumption was verified graphically.*

Larval instar was negatively associated with mortality hazard (β = −0.167, SE = 0.019, Wald $\chi^2 = 77.78$, $p < 0.001$; HR = 0.846; 95% CI: 0.816–0.878). This hazard ratio indicates that each successive developmental stage reduces the instantaneous probability of dying at any given moment by approximately 15.4%, independently of the concentration applied. Compounded across the full ontogenetic range (L1 to L6), this translates to an overall reduction of approximately 56.5% in instantaneous mortality hazard from the most susceptible to the least susceptible instar — a biologically substantial gradient fully consistent with the $LC_{50}$ and $LT_{50}$ patterns identified in the companion Probit study.

Azadirachtin concentration exerted a positive and independent effect on mortality hazard (β = 0.025, SE = 0.006, Wald $\chi^2 = 15.89$, $p < 0.001$; HR = 1.026; 95% CI: 1.013–1.039). Each 100 µL/L increment in concentration increased the instantaneous mortality risk by 2.6%. This quantifies the dose-dependence of azadirachtin's lethal action in a way that complements the $LC_{50}$ estimates: while $LC_{50}$ describes the concentration needed for 50% mortality at a fixed time point (96 h), the concentration HR describes how much faster (or more intensely) death accumulates in real time as concentration increases.

The non-significant interaction between instar and time in the log-minus-log plots (not shown) confirmed that the proportional hazards assumption was not meaningfully violated, supporting the stability of the reported HR estimates across the observation period. Cox-adjusted survival curves at the mean of covariates (Fig. 4, left panel) showed the expected monotonic decline in survival probability from L1 to L6, while the hazard ratio plot (Fig. 4, right panel) visualizes the compounding reduction in mortality risk across instars, ranging from HR = 1.000 (L1 reference) to HR = 0.434 at L6.

# 4 DISCUSSION

This study demonstrates that time-to-event survival modeling provides a complementary and enriched perspective on azadirachtin toxicity against *S. frugiperda* that conventional fixed-endpoint Probit analysis alone cannot deliver. The core biological finding — a progressive and quantifiable ontogenetic

decline in susceptibility, with a qualitative discontinuity at the L3–L4 transition — is robustly confirmed by three independent analytical approaches: $LC_{50}$ progression (companion Probit study), Kaplan–Meier survival estimation, and Cox proportional hazards regression. This convergence substantially strengthens confidence in the biological interpretation beyond what any single method could provide.

# 4.1 The L3–L4 threshold as a qualitative susceptibility boundary

The most striking quantitative finding is the 38.3 percentage-point reduction in 96 h cumulative mortality between L3 (89.9%) and L4 (51.6%) — a change so large that it qualitatively transforms the expected field outcome of a neem application. Against L1–L3 populations, azadirachtin can be expected to achieve near-complete mortality within the 96 h window at all tested concentrations; against L4–L6 populations, more than half of the individuals may survive the same treatment window. The Cox hazard ratio (HR = 0.846 per instar) provides a single mechanistically interpretable parameter for this gradient: each instar transition reduces the instantaneous mortality risk by ~ 15.4%. This is quantitatively consistent with the 271.3% increase in mean $LT_{50}$ from L3 to L4 reported in the companion Probit study, confirming that the L3–L4 boundary is not merely a statistical artifact but a reproducible and biologically meaningful developmental transition.

The physiological basis of this threshold is attributable to cumulative ontogenetic changes in body mass, cuticular architecture, and detoxification enzyme systems. As *S. frugiperda* larvae advance beyond L3, body mass increases substantially, diluting the effective internal dose of ingested toxin per unit body weight. Progressive cuticle thickening and sclerotization restrict transdermal absorption of lipophilic terpenoids such as azadirachtin.[10] Critically, transcriptome-wide analyses of *S. frugiperda* have demonstrated that genes encoding cytochrome P450 monooxygenases, glutathione S-transferases, and ABC transporters are developmentally upregulated in later instars,[11] providing enhanced metabolic clearance capacity that directly attenuates azadirachtin's antifeedant and endocrine-disrupting effects. Additionally, azadirachtin's principal molecular target — the nuclear receptor HR3 in the prothoracic gland, which regulates ecdysone biosynthesis — is most active in instars undergoing frequent molting cycles.[12] Late instars preparing for pupation show reduced HR3-dependent signaling, potentially diminishing azadirachtin's disruptive impact on the hormonal cascade governing larval development.

# 4.2 Temperature and high-altitude context

A theoretically important aspect of our findings is that the ontogenetic susceptibility gradient is fully preserved — and the absolute susceptibility of early instars remains high — under a cooler thermal regime (17°C) than is typical in published azadirachtin bioassays. Temperature modulates insect metabolic rates, feeding behavior, and enzymatic activity in complex ways: lower temperatures reduce feeding rate and metabolic turnover, which could in principle slow toxin ingestion and systemic distribution, but also retard detoxification enzyme activity.[5] The high event rates for L1–L3 (87.9–89.9%) under 17°C conditions suggest that, for early instars, the hormonally disruptive effects of azadirachtin

are sufficiently potent to overcome any temperature-mediated attenuation of toxicokinetics. For late instars, the already-elevated detoxification capacity combined with reduced thermal metabolic rate may compound the resistance phenotype, consistent with the lower event rates observed (42.7–51.6%). These findings suggest that temperature alone does not explain the ontogenetic susceptibility gradient — developmental physiology is the dominant driver — but that the interaction between temperature and instar warrants further investigation under controlled thermostat experiments comparing 17°C and 25°C conditions.

# 4.3 Methodological contributions of survival analysis

From a methodological standpoint, this study illustrates three specific advantages of survival analysis over conventional toxicological endpoints for characterizing botanical insecticide toxicity. First, the Kaplan–Meier estimator correctly handles right-censored data (individuals surviving to 96 h), which conventional ANOVA-based approaches must either exclude or treat as the maximum observed time — a distortion that biases parameter estimates upward for late-instar, lower-susceptibility groups. In our dataset, 48.4–57.3% of L4–L6 observations were right-censored; ignoring censoring would substantially overestimate apparent susceptibility for these groups. Second, the Cox hazard ratio provides a measure of susceptibility that is inherently time-integrated, capturing the differential rate of mortality accumulation across the full 96 h window rather than a single snapshot. The HR = 0.846 per instar is directly comparable across studies that use different observation periods, provided the proportional hazards assumption holds — a key advantage for meta-analyses of larval susceptibility data. Third, the combination of log-rank testing and Cox regression demonstrated that concentration differences that appear large in raw mortality percentages (e.g., 550 vs. 1,667 µL/L) are not necessarily the most discriminating in terms of mortality dynamics, as the log-rank statistic was highest at 550 µL/L — the intermediate concentration — suggesting a non-monotonic sensitivity of the test at extreme concentrations.

# 4.4 Applied implications for high-altitude IPM

The combined Probit and survival analysis findings converge on a single, operationally actionable conclusion for IPM practitioners in Andean highland maize systems: neem-based bioinsecticide applications should be timed to target L1–L3 larval populations. Against these early instars at 17°C, azadirachtin (at concentrations ≥ 275 µL/L of 1.2% EC formulation) will achieve near-complete mortality within 96 h. Against L4–L6 populations, the same application is expected to kill fewer than half the individuals within the same window, regardless of concentration — a failure mode that has direct economic and resistance management implications. The Cox model's concentration HR (1.026 per 100 µL/L) quantifies the diminishing returns of increasing dose: to compensate for a one-instar increase in developmental age, a grower would need to increase concentration approximately 6-fold, which is economically and environmentally untenable.

In Andean highland agroecosystems, where the total larval period of *S. frugiperda* under 17°C conditions may extend to approximately 39 days (compared to ~ 20 days at 25°C), the wider developmental window

provides growers with a longer opportunity to detect and treat early-instar infestations. Systematic whorl scouting beginning at crop emergence, with action thresholds based on L1–L2 instar presence, would maximize the efficacy of azadirachtin-based management. Combination with conservation of larval parasitoids — which similarly exploit the L1–L3 susceptibility window in this same agroecosystem[13] — could provide synergistic biological and chemical control within a cohesive IPM program.

# 5 CONCLUSIONS

Survival analysis of azadirachtin toxicity against all six larval instars of *S. frugiperda* under high-mountain conditions (17°C, 2,586 m a.s.l.) revealed a consistent and quantifiable ontogenetic decline in susceptibility. Kaplan–Meier estimation demonstrated that early instars (L1–L3) achieved 87.9–89.9% mortality within 96 h, while more than half of late-instar (L4–L6) individuals survived the same exposure period. The log-rank trend test confirmed a highly significant dose-dependent effect across the full concentration range ($\chi^2 = 119.35$, $p < 0.001$). Cox proportional hazards regression quantified a 15.4% reduction in instantaneous mortality risk per instar (HR = 0.846; 95% CI: 0.816–0.878) and a 2.6% increase in risk per 100 µL/L concentration increment (HR = 1.026; 95% CI: 1.013–1.039). The convergence between survival-based and Probit dose–response analyses confirms the ontogenetic susceptibility gradient as a robust biological phenomenon rather than a statistical artifact, and substantiates the L3–L4 transition as a critical IPM decision boundary. Neem-based applications in Andean highland maize should be timed to target L1–L3 instars to achieve economically effective control within the biologically relevant 96 h exposure window.

# Declarations

### Ethical Approval

This study used laboratory-reared Spodoptera frugiperda (Lepidoptera: Noctuidae). No vertebrate animals or human participants were involved. Ethical approval is not applicable under current national regulations.

# Conflicts of Interest

The authors declare no conflicts of interest.

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# Author Contribution

H.G.-V. conceived the study, designed the survival analytical framework, performed all statistical analyses (SPSS), interpreted the results, and is solely responsible for the intellectual content and original writing of this manuscript. M.Y.R.-P. and Z.V.C.-M., as undergraduate members of the Semillero de Investigación en Sanidad Vegetal Sustentable, maintained the S. frugiperda laboratory colony, conducted the bioassays, recorded time-to-event data, and contributed to data curation under the direct supervision of H.G.-V. All authors reviewed and approved the final manuscript.

# Data Availability

The time-to-event dataset supporting this study is available from the corresponding author upon reasonable request.

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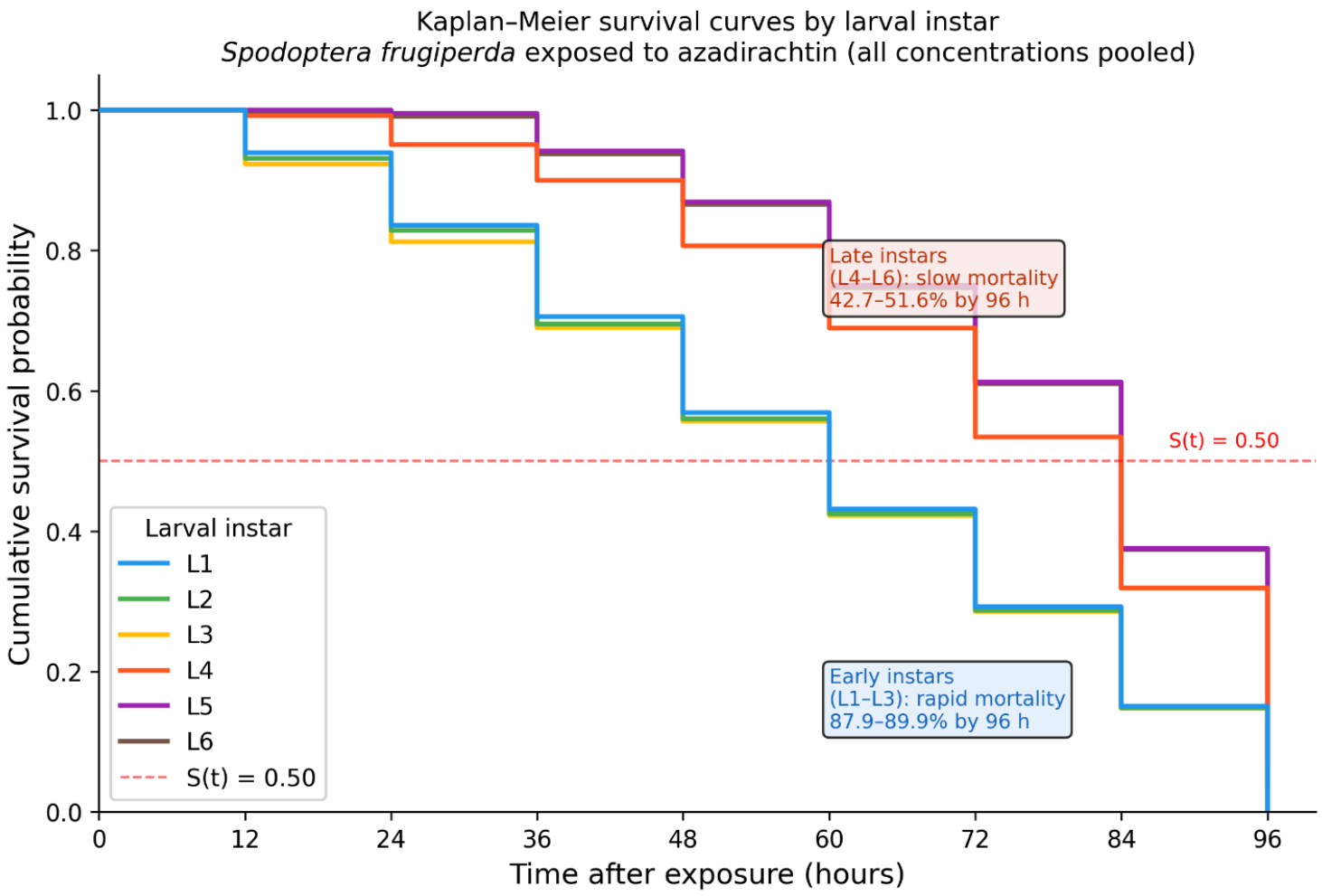
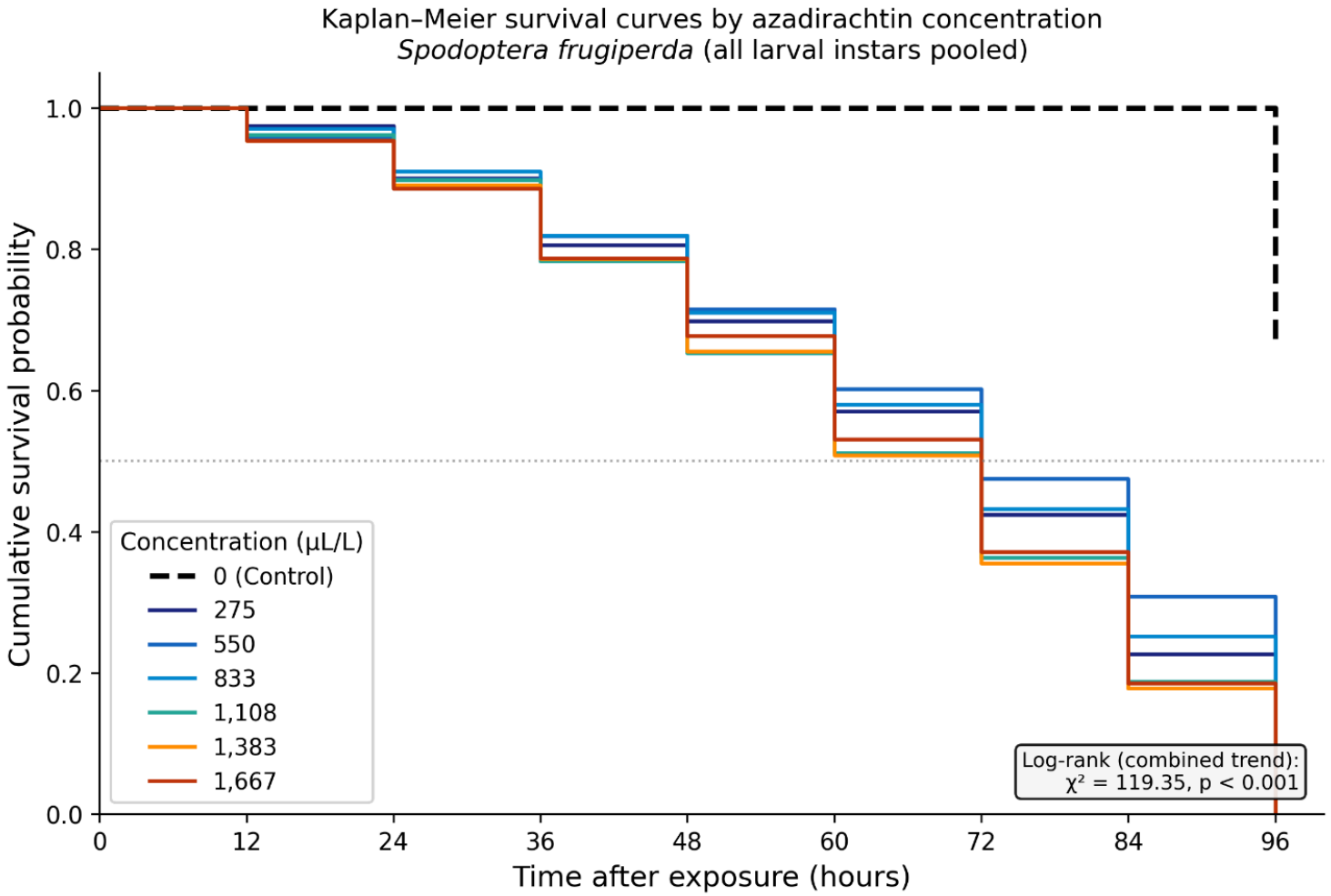
# Figures



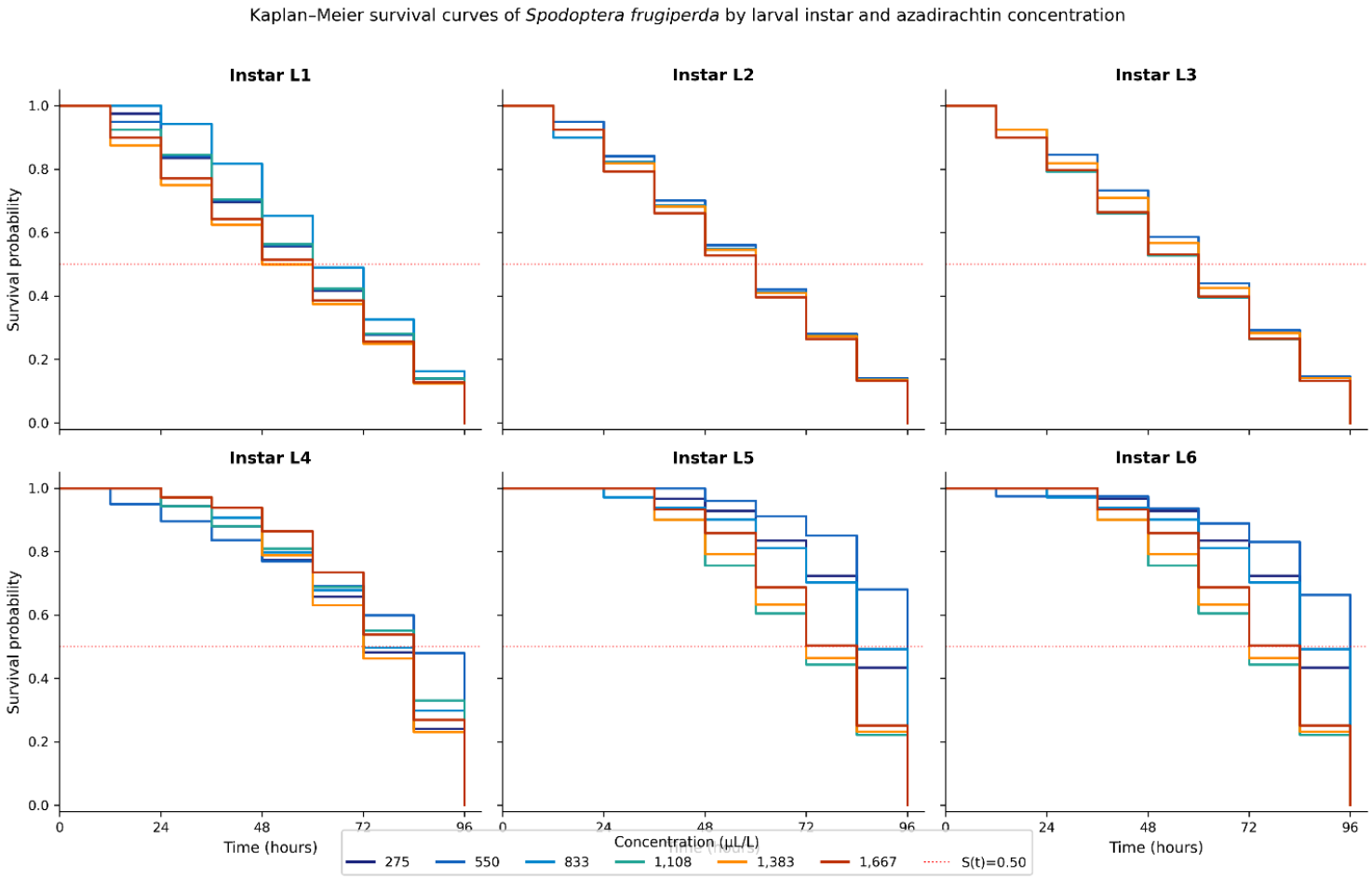
## Figure 1

Kaplan–Meier survival curves for six larval instars of *Spodoptera frugiperda* exposed to azadirachtin under high-mountain laboratory conditions (2,586 m a.s.l.; 17 ± 1°C), with all azadirachtin concentrations pooled within each instar. Each step function represents the product-limit estimate of survival probability over 96 h of observation. Curves for early instars (L1–L3, blue/green tones) show rapid mortality reaching terminal survival of 0.10–0.12, while late instars (L4–L6, warm tones) maintain substantially higher survival (0.48–0.57). The red dashed line indicates S(t) = 0.50 (median survival). Log-rank tests confirmed highly significant differences among all instar pairs ($p < 0.001$).



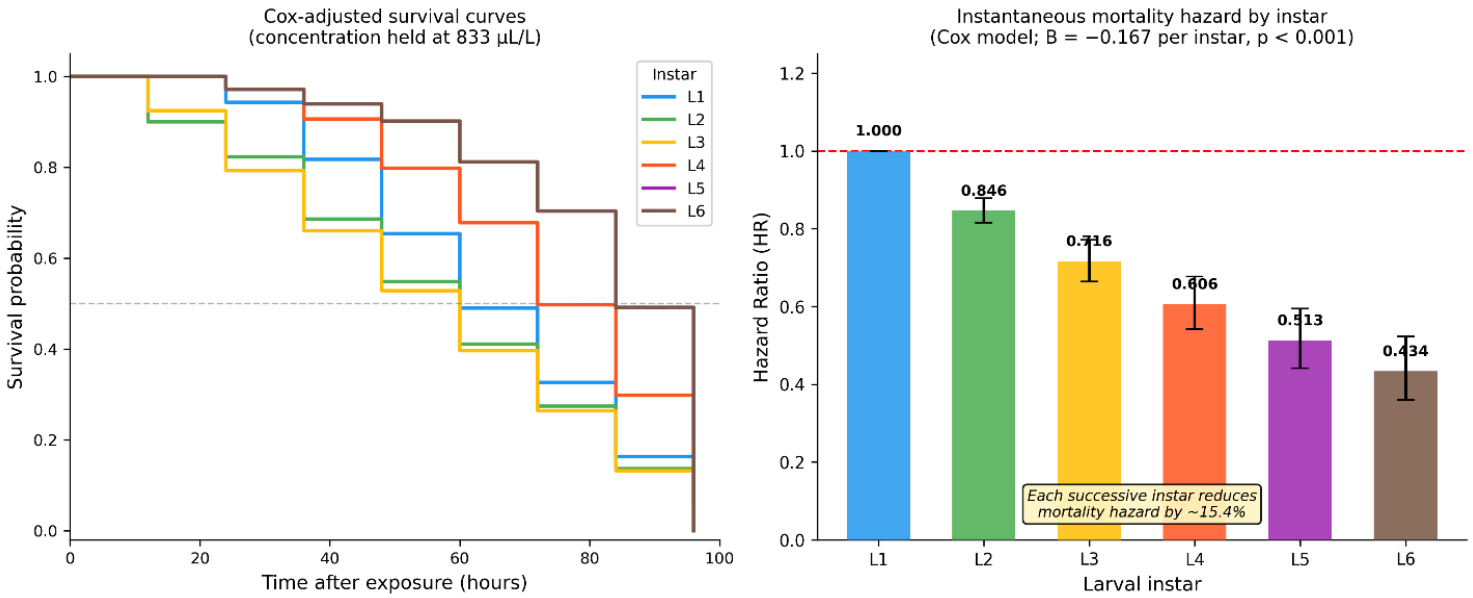

## Figure 2

Kaplan–Meier survival curves stratified by azadirachtin concentration (0–1,667 µL/L of 1.2% EC formulation) for all larval instars of *S. frugiperda* pooled. The control group (0 µL/L, dashed black line) maintains near-complete survival throughout 96 h. All treated concentrations (275–1,667 µL/L) produced significant reductions relative to controls (log-rank $p < 0.001$). The combined concentration trend test was highly significant ($\chi^2 = 119.35$, df = 1, $p < 0.001$), confirming a dose-dependent toxicological effect.




## Figure 3

Kaplan–Meier survival curves of *S. frugiperda* by larval instar (A–F: L1 to L6) and azadirachtin concentration (275–1,667 µL/L). Each panel shows instar-specific survival dynamics across concentrations. Note the qualitative difference between early instars (A–C), where all concentrations produce steep survival declines converging to low terminal survival, and late instars (D–F), where curves are flatter, more compressed, and show persistently higher terminal survival probabilities even at maximum concentration. The red dotted line in each panel indicates S(t) = 0.50.




## Figure 4

Left: Cox-adjusted survival curves by larval instar with azadirachtin concentration held at 833 µL/L (nearest to mean). Right: Hazard ratios (HR) derived from the Cox model (β = −0.167 per instar; HR = 0.846 per instar step) for instars L1–L6, with L1 as reference (HR = 1.000). Error bars represent 95% confidence intervals. Each successive instar reduces instantaneous mortality hazard by approximately 15.4%, with L6 showing an estimated 56.6% reduction in mortality hazard relative to L1.