Strength of enemy release from parasitoids is context-dependent in the invasive African Fig Fly, *Zaprionus indianus*

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Abstract
Understanding the mechanisms underlying the success of biological invasions is essential to employ effective prediction and management strategies. The escape from natural enemies in invaded regions (enemy release hypothesis) and increased competitive ability are hallmarks of invasive species; however, these two processes are rarely studied within the same context. Here, we examined the effect of enemy release on the competition outcomes of a successful invasive insect pest in North America, the African fig fly (*Zaprionus indianus)*. Parasitoid wasps such as *Leptopilina heterotoma* that parasitize drosophilid larvae may seek out established species with known host suitability over a novel species, so we hypothesized *Z. indianus* may have low susceptibility to parasitoids, giving them a competitive advantage over co-occurring drosophilids. We tested this hypothesis by comparing the adult emergence rates from *Z. indianus* larvae reared alone or in competition with *Drosophila hydei* or *D. simulans* larvae in the presence and absence of parasitoid wasps. These interactions might be influenced by larval density, so we tested competitive interactions under low and high larval densities. At low larval densities, *Z. indianus* emerged at equal rates to *D. hydei* but outcompeted *D. simulans,* and these outcomes were not affected by parasitoids. However, at high densities, the addition of parasitoids shifted competition outcomes in favor of *Z. indianus*, suggesting enemy release provides a competitive advantage under some circumstances. These results indicate that the strength of enemy release in *Z. indianus* is widely dependent on contextual factors such as density and competitor species. Further investigation of how these results apply to field environments could offer insight into how *Z. indianus* alters ecosystems and how productive biological control may limit the spread of *Z. indianus*.

Short Abstract
Invasive species may succeed in new environments in part because they are less susceptible to diseases and parasites that have co-evolved with local hosts, giving invaders a competitive advantage. We tested this hypothesis by competing an invasive fruit fly against established species in the presence of parasitoid wasps that lay their eggs in fruit fly larvae. We found that the invasive species generally outcompeted other species in the presence of parasitoids, but the extent of its advantage depended on the species it was competing against and the number of larvae present.

Introduction

The spread of invasive species poses significant ecological, environmental, and economic threats. Invasive species have largely contributed to biodiversity loss and the extinction of native species (Bradshaw et al. 2016) and are destructive in non-native environments, leading to agricultural damage and economic costs (Diagne et al. 2021). Furthermore, invasive species raise human health concerns as they can alter disease-vector dynamics and facilitate the spread of pathogens (Roy et al. 2022). Despite the well-known risks of biological invasions, managing invasive species remains a challenge. A comprehensive understanding of the mechanisms underlying invasive species' success is vital for effective conservation, management, and mitigation practices.

One of the most widely proposed hypotheses explaining the success of invasive species is the enemy release hypothesis (Keane & Crawley 2002, Enders et al. 2018). This hypothesis proposes that invasive species are released from their natural enemies in non-native environments, resulting in population growth of the invader as native species remain restrained by their natural enemies. Competition with native species also plays a crucial role in determining the success or failure of invasive species (Clarke & McGeoch 2023). The competitive ability of invasive species is often superior to native species, facilitating their dominance in invaded communities (Gioria & Osborne 2014). Although components of enemy release and competition overlap, there are few studies examining both as factors for invasion success. Studying these two processes within the same context may provide a more cohesive understanding of the spread and success of invasive species.

The African fig fly, *Zaprionus indianus*, is part of the Drosophilidae family and is native to tropical regions of central Africa. In the 1990s, *Z. indianus* was first detected in South America in Brazil, where it quickly spread across the continent (Vilela 1999). Following a northward invasion pattern, *Z. indianus* was detected in Florida in 2005 (Van der Linde et al. 2006) and has since been detected across eastern North America (Pfeiffer et al. 2019, Rakes et al. 2023). Sampling data from orchards reveal repeated and rapid population growth relative to other drosophilids, suggesting *Z. indianus* outcompetes established drosophilid species (Rakes et al. 2023). However, the factors that facilitate the success of *Z. indianus* remain unknown. Evaluating the role of enemy release in competition outcomes could shed light on the mechanisms underlying the repeated success of *Z. indianu*s in novel environments. *Zaprionus indianus* is an optimal study system as its relatively recent arrival to North America and lack of permanent establishment in temperate regions (Pfeiffer et al. 2019) allows the opportunity to evaluate the potential loss of attack by native natural enemies. Additionally, *Z. indianus* likely experiences high rates of resource competition with other drosophilid species because it occupies similar niches (e.g., opportunistic oviposition in rotting host fruits) (Bragard et al. 2022).

Parasitoid wasps are natural enemies of drosophilids and exert strong selective pressures on drosophilid populations, with up to 90% of drosophilid parasitization rates (Fleury et al. 2004). Additionally, host-parasitoid interactions exhibit coevolutionary arms races, and each have highly specialized attack and defense mechanisms (Godfray & Shimada 1999). Invasive

species lack this coevolutionary relationship, which raises questions about how they interact with parasitoids of established drosophilids (Kraaijeveld et al. 1998). *Leptopilina heterotoma* is a generalist parasitoid wasp found in North America that parasitizes larvae of numerous drosophilid species due to its low selection threshold (Quicray et al. 2023, de Bruijn et al. 2022). Although *L. heterotoma* is a generalist, not all hosts are suitable for parasitoid development (Quicray et al. 2023). When examining *Z. indianus*' cellular immune response to parasitization, Kacsoh et al. (2014) found that while *L. heterotoma* detects *Z. indianus* as potential hosts (e.g., ovipositing in larvae), the majority of *Z. indianus* successfully emerged as adults, suggesting that the immune system of *Z. indianus* is resistant to *L. heterotoma*. Additionally, no *L. heterotoma* adults emerged from *Z. indianus*, indicating *Z. indianus* is an unsuitable host.

Resource competition is another major driving force of natural selection (Bernhardt et al. 2020). Ephemeral food substrates, such as rotting fruits and their associated microbiota, result in both intra- and interspecific competition for drosophilids (Rohlfs & Hoffmeister 2004). Competition outcomes in drosophilids are widely context-dependent and may fluctuate based on abiotic and biotic factors such as air temperature or fruit substrate (Comeault & Matute 2021, de Paiva Mendonça 2023). Host-parasitoid interactions can also influence competition outcomes. For example, *Drosophila melanogaster* outperforms sibling species *D. simulans* in the absence of parasitoids, but these competition outcomes are inverted when parasitoids are present (Boulêtreau et al. 1991). However, it is unknown how parasitoids may alter competition outcomes between invasive and established drosophilid species. If *L. heterotoma* preferentially parasitizes established species in the presence of *Z. indianus*, then *Z. indianus* may capitalize on reduced competition stressors.

In this study, we evaluated how parasitoids affect the competition outcomes of *Z. indianus* under controlled laboratory conditions. As our main goal was to evaluate how enemy release influences the dominance of *Z. indianus* in invaded environments, we used a community-comparison approach as described by Brian & Catford (2023). We measured adult emergence rates in interspecific and intraspecific competition assays in the absence and presence of parasitoid wasps to compare competition outcomes both between and within species. In the interspecific competition assays, we offered parasitoids the opportunity to parasitize the larvae of *Z. indianus* and another drosophilid species. We used *D. simulans* and *D. hydei* as co-occurring established species as they are both cosmopolitan in distribution (Werner et al. 2018). Given the lack of coevolutionary history and presumed host incompatibility of *Z. indianus*, we hypothesized that *Z. indianus* would be less susceptible to parasitoids than established drosophilids, leading to higher emergence rates for *Z. indianus*. Specifically, we aimed to answer three questions: 1) Does *Z. indianus* outcompete established species in the absence of parasitoids? 2) Does the presence of parasitoids alter competition outcomes to mediate *Z. indianus* success? 3) Does larval density influence the degree to which parasitoids alter competition outcomes? Given the rapid population growth of *Z. indianus* observed in orchards (Rakes et al. 2023), we predicted that *Z. indianus* performs equal to or better than established

species in the absence of parasitoids, and this advantage is further intensified by the presence of *L. heterotoma*, alleviating competition stressors for *Z. indianus*.

Materials & Methods

Insect rearing

Adult *Z. indianus* were obtained from isofemale lines derived from females collected in North America in 2022 and 2023 (Rakes et al. 2023). *Drosophila simulans* were collected from orchards in Florida in 2022, and *D. hydei* were collected from orchards in Virginia in 2023. All flies were maintained on a cornmeal-molasses artificial diet at room temperature. All parents of the experimental offspring were raised in controlled lab conditions for at least two generations. *Leptopilina heterotoma* (Lh14) stocks were obtained from Todd Schlenke at the University of Arizona. The Lh14 strain was originally collected in 2002 from Winters, California (Kacsoh & Schlenke 2012). Parasitoids were maintained on laboratory strains of *D. melanogaster* (w1118 and Canton-S). To elongate parasitoid lifespan, 500 µL of 50% honey-water solution was provided on vial plugs.

Intraspecific competition assays

To test how parasitoid presence affects intraspecific competition outcomes of *Z. indianus,* we prepared vials with only *Z. indianus* larvae in the absence and presence of parasitoid wasps. We compared emergence rates at low densities (50 total larvae) and high densities (150 total larvae) to account for the varying fly densities observed in the field across different locations and time periods (Rakes et al. 2023). To collect larvae, we allowed adult flies to oviposit on 3% agar plates containing 10% grape juice concentrate for 24 hours. A paste of baker's yeast and water was added to each plate to encourage oviposition. We collected two-day-old larvae from the plates and inoculated vials filled with 10 mL of cornmeal-molasses substrate. For parasitized treatments, we immediately added six adult *L. heterotoma* (three female, three male) to the inoculated vials for 24 hours. Parasitoids were not offered hosts prior to experiments. In the control treatments, we followed the same procedure but did not add parasitoids. We counted emerging adult flies every 1-2 days until no further adults emerged. Intraspecific assays had two replicates per treatment. We conducted two independent intraspecific assays (one alongside each interspecific assay, see below) and combined the data for a total of four vials per density and parasitoid treatment.

Comparing parasitoid susceptibility across Z. indianus *populations*

Genomic data suggest that despite a population bottleneck, introduced North American *Z. indianus* populations retain substantial genetic variation relative to congeners (Comeault et al. 2020 & 2021). To test for potential genetic differences in parasitoid susceptibility, we compared emergence rates of *Z. indianus* from five Florida (FL) isofemale lines, and four Connecticut (CT) isofemale lines collected in 2022 (Rakes et al. 2023). We chose these lines as they had the largest

geographic separation of any lines available in our lab. These isofemale lines were reared in the lab for approximately 16 months (~20 generations) prior to the experiments described here. We added a total of 25 *Z. indianus* larvae to each vial. We prepared three replicates of parasitized vials for each line, resulting in a total of 27 vials. Two parasitoids (one female, one male) were added to vials for 24 hours.

Interspecific competition assay

To test how parasitoid presence affects interspecific competition outcomes, we compared the larva to adult survival rates of *Z. indianus* and co-occurring drosophilid species (*D. hydei or D. simulans)* reared together in the absence and presence of parasitoid wasps. Low and high total total densities were the same as the intraspecific assays (50 and 150 larvae); however, interspecific assays contained equal numbers of each species. We collected larvae as described above from the two species separately and combined them into a single vial with or without parasitoids. We added six parasitoids (three female, three male) for 24 hr. We counted adults of both species every 1-2 days as they emerged. Each interspecific treatment had five replicates, totaling 20 vials per species comparison. All experiments were incubated at room temperature.

Assessing potential parasitoid host-switching

In interspecific conditions, evaluating emergence rates with differing parasitoid exposure times may reveal whether *L. heterotoma* exhibits host-switching (Murdoch 1969), in which a parasitoid initially oviposits in a favorable host-species, but then switches to a different host after the original host becomes saturated. As *D. simulans* is a known suitable host of *L. heterotoma* (Quicray et al. 2023), we predicted that *L. heterotoma* might initially oviposit in *D. simulans* before parasitizing *Z. indianus*. To assess parasitoid foraging behavior on the interspecific outcomes of *Z. indianus* and *D. simulans*, we conducted a competition assay using various parasitoid exposure times. Here, we compared the emergence rates of *Z. indianus* and *D. simulans* following 1 hr, 8 hr, and 24 hr of parasitoid exposure. All vials contained a low density of 20 larvae (10 of each species) to increase the likelihood of host saturation. Two parasitoids (one female, one male) were added to parasitized treatments. Non-parasitized treatments contained no parasitoids and were indicated by 0 hr of parasitoid exposure. Each parasitoid duration treatment had three replicates (9 total vials), and the non-parasitized controls had 12 replicates.

Data Analysis

We used the proportion of adult flies relative to starting larvae to calculate emergence rates of each treatment. We used a generalized linear model (GLM) with binomial errors to compare *Z. indianus* emergence rates for the intraspecific assays. Emerged adult flies were modeled as a function of density, parasitoid presence, and a density*parasitoid interaction. We used a generalized linear mixed model (GLMM) with binomial errors to compare *Z. indianus* emergence rates from Florida (FL) and Connecticut (CT) populations. The model included

location as a main effect and isofemale line as a random effect. We analyzed the interspecific data in separate models for each density and species comparison. We used GLMs with quasibinomial errors to compare species emergence rates between treatments. The quasibinomial errors corrected for overdispersion in the data. Emerged adult flies were modeled as a function of species, parasitoid presence, and a species*parasitoid interaction. We used a GLM with binomial errors to assess differences in *Z. indianus* and *D. simulans* emergence rates at various parasitoid exposure times as a function of the species, exposure time, and a species*exposure time interaction. We calculated estimated marginal means for each model and performed pairwise linear contrasts between experimentally relevant groups using the *emmeans* package (Lenth et al. 2020) in R. We generated Tukey-adjusted linear contrast P values; statistical significance was determined by $P_{adjusted} < 0.05$. Only the results of relevant contrasts are reported. All analyses were performed using the R statistical software (v4.4.0; R Core Team 2024). All plots were generated with *ggplot* (Wickham 2016) and data were managed with *data.table* (Dowle & Srinivasan 2012).

Results

Intraspecific competition assay

For intraspecific assays, we compared the emergence rates of *Z. indianus* at low and high densities in the absence and presence of *L. heterotoma* (Fig. 1, Appendix Table 1). High density (GLM; Z = -8.326, P < 0.001) and presence of parasitoids (GLM; Z = -8.600, P < 0.001) significantly decreased *Z. indianus* emergence rates.

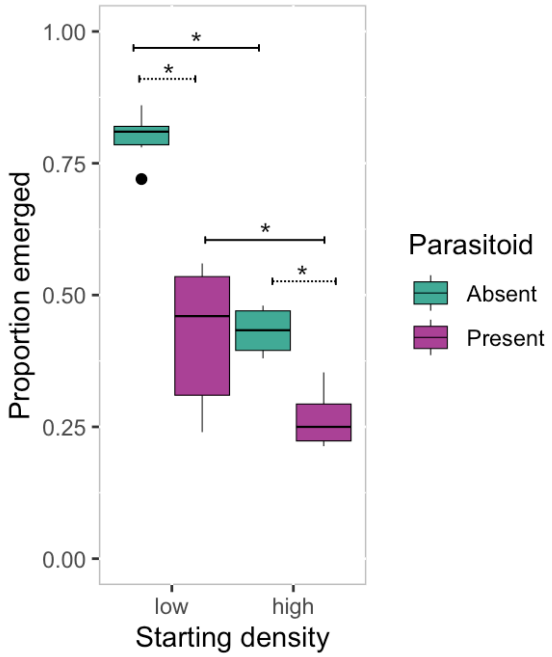

Figure 1: *Z. indianus* is susceptible to parasitism by *L. heterotoma*. Intraspecific competition assays measuring *Z. indianus* emergence rates in the presence and absence of *L. heterotoma*. Flies were reared at two densities: low (50 total larvae) and high (150 total larvae), and emergence rate is the proportion of adults relative to starting larvae. Asterisks indicate statistical significance by linear contrast of a generalized linear model ($P_{adjusted} < 0.05$). Solid lines with asterisks indicate significant differences across densities. Dotted lines with asterisks indicate significant effects of parasitoids within densities. N=4 replicate vials per treatment; data are combined from two independent intraspecific competition experiments with two replicates per treatment.

Comparing parasitoid susceptibility across Z. indianus *populations*

Because the intraspecific assay revealed higher parasitization rates of *Z. indianus* than previously reported (Kacsoh et al. 2014), we tested whether genetic variation within *Z. indianus* lines might lead to different susceptibilities to *L. heterotoma.* Despite variation within and between isofemale lines, there were no significant differences in adult emergence rates between parasitized Connecticut and Florida populations (Figure 2; Appendix Table 1; GLMM; P = 0.561).

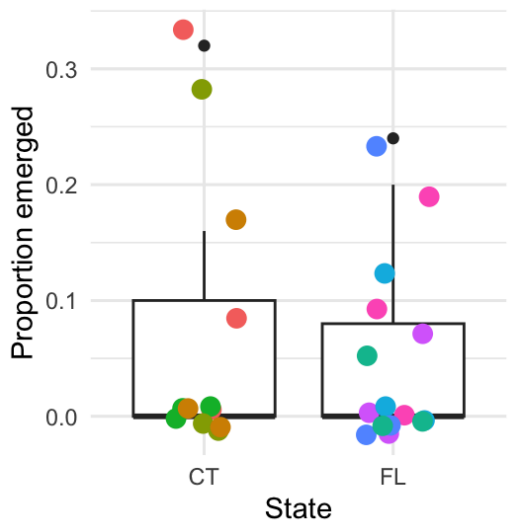

Figure 2: No geographic variation in *Z. indianus* susceptibility to *L. heterotoma*. Adult emergence rates from parasitized Connecticut (CT) and Florida (FL) *Z. indianus* isofemale lines. Boxplots indicate combined emergence rates of all lines and replicates from each state. Points indicate individual replicate vials; colors indicate unique isofemale lines from CT or FL. N=4 lines for CT and N=5 lines for FL with three replicates of 25 larvae per line.

Interspecific competition assay

For the interspecific competition assays, we first compared the adult emergence rates of *Z. indianus* and *D. hydei* reared together at low and high density and in the absence or presence of parasitoids (Fig. 3A, Appendix Table 1). At low density, parasitoid presence significantly decreased the emergence rates of *Z. indianus* and *D. hydei* (linear contrast; Z = 4.234, P < 0.001 & Z = 2.837, P = 0.024, respectively). However, there were no significant differences between *Z. indianus* and *D. hydei* emergence rates in either the absence or presence of parasitoid wasps (linear contrast; Z = -0.654, P = 0.914 & Z = -1.748, P = 0.299, respectively). At high density, the presence of parasitoids significantly decreased the emergence rates of *D. hydei* (linear contrast; Z = 7.402, P < 0.001), but did not affect *Z. indianus* emergence rates (linear contrast; Z = 0.061, P = 0.999). *Z. indianus* had significantly lower emergence rates than *D. hydei* in the absence of parasitoids (linear contrast; Z = 5.445, P < 0.001), but had significantly higher emergence rates in the presence of parasitoids (linear contrast; Z = -4.383, P < 0.001).

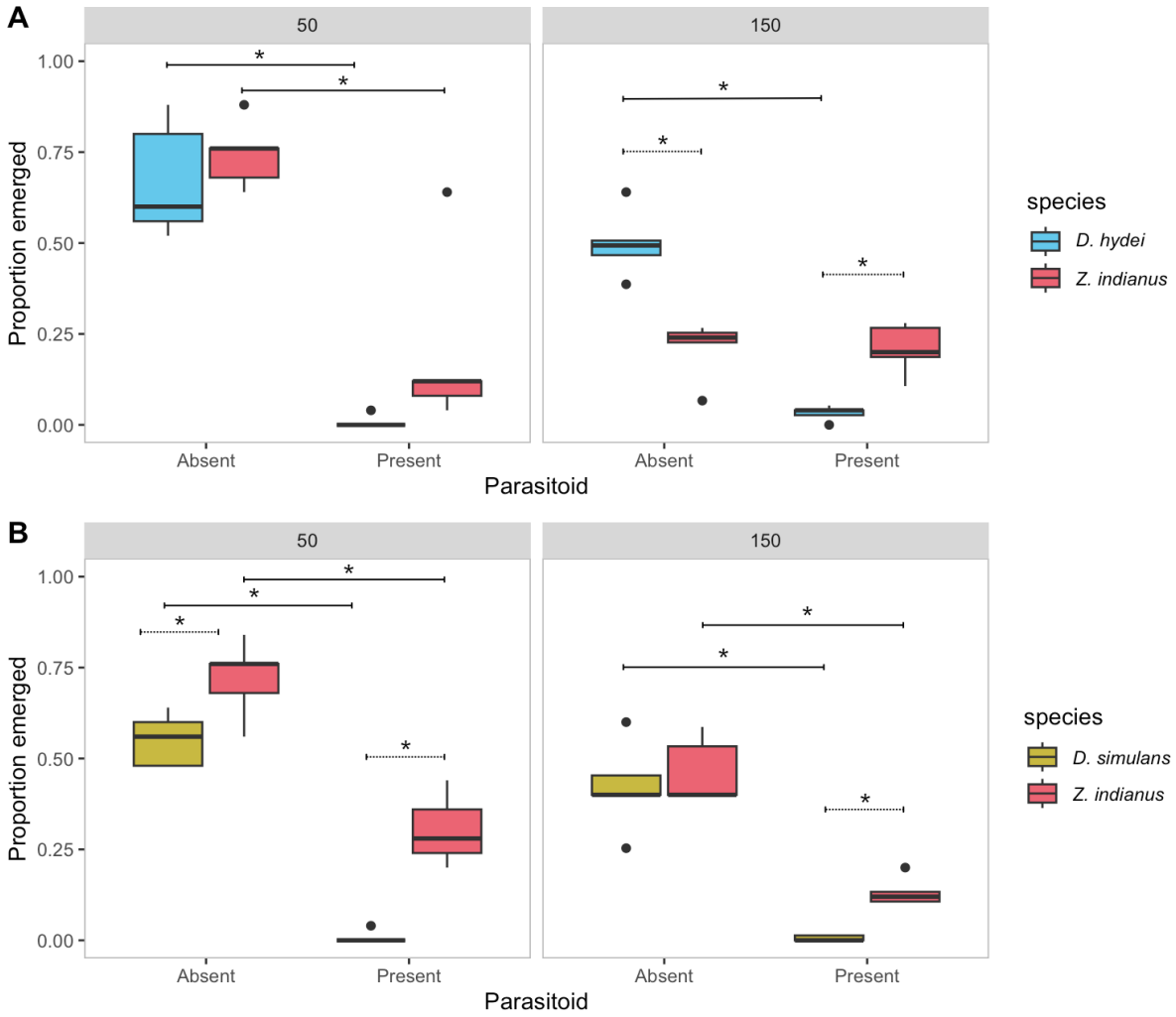

Figure 3: Enemy release from *L. heterotoma* depends on competing species and density in *Z. indianus*. A) Interspecific competition assay results comparing *Z. indianus* and *D. hydei* and B) *Z. indianus* and *D. simulans* adult emergence rates. Gray labels indicate density: the low density is 50 total larvae (25 of each species) and the high density is 150 total larvae (75 of each species). Asterisks indicate statistical significance by linear contrast of a generalized linear model ($P_{adjusted} < 0.05$). Solid lines with asterisks indicate significant effects of parasitoids within species. Dotted lines with asterisks indicate significant differences between species raised in direct competition. N=5 replicate vials per treatment.

Second, we observed the emergence rates of *Z. indianus* and *D. simulans* reared under the same set of conditions as the *D. hydei* experiment (Fig. 3B, Appendix Table 1). At low density, parasitoid presence significantly decreased the emergence rates of *Z. indianus* and *D. simulans* (linear contrast; $Z = 6.360$, $P < 0.001$ & $Z = 4.924$, $P < 0.001$, respectively). However, *Z. indianus* emergence rates were significantly higher than those of *D. simulans* in both the absence and presence of parasitoids (linear contrast; $Z = -2.737$, $P = 0.032$ & $Z = -3.898$, $P < 0.001$). At

high density, parasitoid presence also significantly decreased the emergence rates of both *Z. indianus* and *D. simulans* (linear contrast; Z = 6.288, P < 0.001 & Z = 4.586, P < 0.001, respectively). There were no significant differences between *Z. indianus* and *D. simulans* emergence rates in the absence of parasitoids (linear contrast; Z = -0.787, P = 0.861). However, *Z. indianus* had significantly higher emergence rates than *D. simulans* in the presence of parasitoids (linear contrast; Z = -3.098, P = 0.011).

Host switching assay

To assess potential effects of parasitoid exposure time on the interspecific competition outcomes of *Z. indianus* and *D. simulans*, we evaluated mean emergence rates with 1 hr, 8 hr, and 24 hr parasitoid exposure times (Fig. 4; Appendix Table 1). Exposure to parasitoids for one hour significantly decreased the emergence rates of *D. simulans* (linear contrast; Z = -3.819, P < 0.001), but not *Z. indianus* (linear contrast; Z = -1.560, P = 0.119), when compared to non-parasitized controls. However, exposure to parasitoids for at least 8 hours significantly decreased the emergence rates of both *D. simulans* and *Z. indianus* (linear contrast; P < 0.05 for all comparisons).

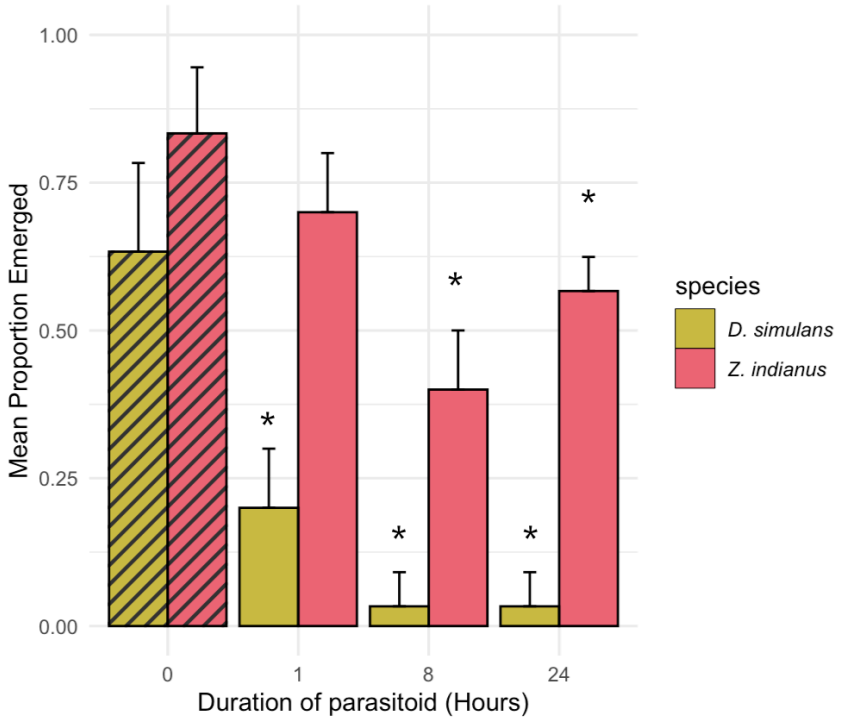

Figure 4: *L. heterotoma* parasitizes *D. simulans* more rapidly than it parasitizes *Z. indianus*. Interspecific assay comparing the emergence rates of *Z. indianus* (red bars) and *D. simulans* (yellow bars) at different parasitoid exposure times. Hashed lines represent control vials with no parasitoids added (N=12 total). Parasitized treatments had three replicates for each exposure time. A density of 20 larvae (10 of each species) was used

for all vials. Error bars represent standard deviation. Asterisks indicate significant differences between control and parasitized treatments by linear contrast ($P_{adjusted} < 0.05$).

Discussion

We explored the effect of enemy release on influencing competition outcomes by comparing the adult emergence rates of a recent invasive insect pest, *Z. indianu*s, and co-occurring drosophilid species in the absence and presence of *L. heterotoma* parasitoid wasps. Here, we demonstrate that consideration of competition interactions and environmental context is essential when assessing the relative strength of enemy release. Our results suggest that enemy release in the presence of competing species may contribute to the rapid population growth of *Z. indianus* that is commonly observed in the field, and drosophilid density affects the relative strength of enemy release in benefiting *Z. indianus* populations. We found that under most circumstances, *Z. indianus* is an equal or better competitor than *D. hydei* and *D. simulans*, but competition outcomes are widely dependent on factors such as parasitoid presence, density, and competitor species. Importantly, under the most stressful conditions (high density and parasitoids present), *Z. indianus* outcompeted both species.

By comparing the emergence rates of *Z. indianus* with co-occurring drosophilid species in the absence and presence of parasitoids, our study demonstrates that parasitoid presence had neutral or positive effects on *Z. indianus* interspecific competition outcomes. Our findings support the enemy release hypothesis because the emergence rates of *Z. indianus* were equal to or higher than *D. hydei* and *D. simulans* in the presence of parasitoids (Fig. 3), indicating that parasitoids were more detrimental to the established species than to *Z. indianus*. However, we also observed that parasitoids significantly decreased the emergence rates of *Z. indianus* in intraspecific assays (Fig. 1), and *Z. indianus* emergence rates were never higher than 50% in the presence of parasitoids. These results differ from the study by Kacsoh et al. (2014), which reported the majority (> 50%) of *Z. indianus* escaped parasitism by *L. heterotoma*, but they are similar to a study by Cinege et al. (2023) which reported fewer than 30% of *Z. indianus* emerged in the presence of *L. heterotoma*. It is unlikely that genetic variation between *Z. indianus* lines is responsible for these differences, as we found no reproducible variation in emergence rates across isofemale lines (Fig. 2). Therefore, parasitoids may be interpreted as generally beneficial for *Z. indianus* when co-occurring drosophilid species are present (e.g., *Z. indianus* commonly outperformed co-occurring species), but detrimental in intraspecific conditions (e.g., *Z. indianus* emergence rates were significantly decreased compared to non-parasitized controls). In addition to physiological defense mechanisms, behavioral responses are also vital for reducing parasitism in hosts (Hart 1990). Assessing whether the presence of parasitoids or competing species can influence *Z. indianus'* oviposition or larval foraging behavior may provide insights into potential behavioral resistance strategies of *Z. indianus* in field environments. In sum, *Z. indianus* benefits from enemy release when competing with co-occurring drosophilids despite still being

susceptible to parasitoids.

Our findings further suggest that the benefit of enemy release to *Z. indianus* is density-dependent. At low densities, the addition of parasitoids did not alter competition outcomes for *Z. indianus* with *D. hydei* or *D. simulans*. Therefore, at low densities, the parasitoids have approximately equal effects on both competing species and do not alter competition dynamics. However, at high densities, we observed that parasitoid presence shifted the competition outcomes in favor of *Z. indianus* in both interspecific assays. Since competition outcomes switched to benefit *Z. indianus* at high densities, but not low densities, we conclude that enemy release was stronger at high densities. A possible explanation for differing competition outcomes based on density is that *L. heterotoma* switched from a known host (D. *simulans* or *D. hydei*) to a novel host (*Z. indianus)*. As host switching is likely initiated by optimal foraging behavior (Křivan 1996), an increase in the abundance of suitable hosts (e.g., high density) might decrease the number of parasitized suboptimal hosts.

We tested the possibility of host switching by examining the effects of parasitoid exposure time on the emergence rates of *Z. indianus* and *D. simulans* (Fig. 4). Our data suggest parasitoids initially oviposited in *D. simulans,* but switched to *Z. indianus* once suitable hosts were saturated. The difference in parasitization rates might result from variations in behavioral avoidance strategies between *Z. indianus* and *D. simulans* larvae, or *L. heterotoma* may prefer to oviposit in *D. simulans*. Although we did not directly assess parasitoid host preference, parasitoids are highly sensitive to host-selection and can accept or reject hosts based on their suitability and quality (Wertheim 2022). Additionally, *D. simulans* primarily uses lamellocytes for its anti-parasitoid immune response, rather than multinucleated giant hemocytes like *Z. indianus* (Salazar-Jaramillo et al. 2014, Cinege et al. 2020). Virulence factors of *L. heterotoma* have evolved mechanisms to combat the lamellocyte immune response (McGonigle et al. 2017), suggesting that species using lamellocytes are more favorable hosts than species using multinucleated giant hemocytes. Therefore, it is possible that the density-dependent results observed in the interspecific assays reflect host switching behavior of *L. heterotoma*. Although host-switching is commonly observed in generalist parasitoids (Murdoch 1969, Jones et al. 2015), specialist parasitoid species may be more reluctant to oviposit in suboptimal hosts due to increased host selectivity. Therefore, evaluation of how different parasitoid species interact with *Z. indianus* in interspecific conditions would be beneficial for a broader understanding of enemy release since parasitoid life-history strategies may produce different competition outcomes.

All experiments in this study were conducted under controlled laboratory conditions, which may limit applicability to field environments. We used larva to adult emergence rates as an indicator for population success. However, we did not test other fitness components such as growth, fecundity, and reproductive output, which may also be used to assess enemy impact. Assessment of multigenerational effects using multiple fitness factors may more comprehensively determine the impact of parasitoid presence on invader population performance. In *D. melanogaster,* adult females that successfully defended themselves from

parasitism had several reduced fitness traits such as smaller body size and lower fecundity (Fellowes et al. 1999). Additionally, investment in parasitoid defense mechanisms (e.g., increased hemocyte counts) are associated with tradeoffs in competitive ability in *D. melanogaster* (Kraaijeveld et al. 2001). Therefore, it is possible that increased defense mechanisms lead to diminished fitness outcomes for *Z. indianus*, thus decreasing population growth. Further evaluation of how parasitism affects *Z. indianus* population dynamics over time would be beneficial to better understand the strength and frequency of enemy release in the field. Additionally, using a more natural experimental design (e.g., natural fruit substrates, multiple co-occurring drosophilid species) to mirror environmental heterogeneity could provide more information on how these results apply to the field. Specifically, the use of artificial food substrates limited our evaluation of how larval predator avoidance (e.g., escape mechanisms) may alter competition outcomes in parasitized treatments. Other abiotic environmental conditions are also known to influence *L. heterotoma* host selection (Roitberg et al. 1992). Unfavorable conditions such as changes in barometric pressure and humidity may diminish foraging strategies, leading to increased parasitization of suboptimal hosts in the field. Overall, complex and variable field conditions could potentially produce different ecological outcomes than those observed in this study.

Conclusion

The enemy release hypothesis has raised controversy due to its mixed support within and across taxa (Colautti et al. 2004, Liu & Stiling 2006, Brian & Catford 2023). Our findings further emphasizes how varying contexts (e.g., density or competitor species) may alter species interactions and consequently affect the interpretation of enemy release. Here, we demonstrate that enemy release may contribute to the success of *Z. indianus*, and larval density influences the relative strength of enemy release. We confirm that *L. heterotoma* effectively decreases the emergence rates of *Z. indianus*, which may have major implications for management of *Z. indianus* in the field. However, these benefits may be ameliorated when more suitable hosts are present, especially at high larval densities. Due to the increasing threat of *Z. indianus* as an agricultural pest, productive biological control may control the growth and spread of *Z. indianus* populations. Verification of these results in a natural environment would be highly beneficial for understanding how this invasive species interacts with native ecosystems, and for determining if biological control could be an effective strategy for managing *Z. indianus* populations.

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Data availability statement
All raw data used for these analyses and code to replicate the figures and statistics are available at https://doi.org/10.5061/dryad.6hdr7sr8n. Data and code are also available at https://github.com/camille-wa/Zaprionus-parasitoid-experiments.

Competing interests statement

The authors declare no competing interests.

Author contributions

CRW and PAE conceptualized the study and acquired funding. CRW developed methodology, conducted all experiments, collected and analyzed all data, created figures, and wrote the manuscript with supervision from PAE. CRW and PAE revised the manuscript.

Appendix

Appendix Table 1: Generalized linear model results for each experiment. Z-values are reported for all experiments except for the interspecific competition assays (Fig. 3), where T-values are given in italics due to use of quasibinomial errors. Significant terms were determined by $P < 0.05$ and are indicated in bold.

Experiment	Term	Estimate	SE	Z/*T*	P-value
Z. indianus intraspecific (Fig. 1)	Intercept	1.325	0.174	7.632	**< 0.001**
	Parasitoid	-1.966	0.229	-8.600	**< 0.001**
	Density	-1.600	0.192	-8.326	**< 0.001**
	Parasitoid:Density	1.230	0.260	4.729	**< 0.001**
Z. indianus Florida vs Connecticut (Fig. 2)	Intercept	-2.753	0.400	-6.890	**< 0.001**
	State	-0.307	0.528	-0.581	0.561
Z. indianus vs *D. hydei* low density (Fig. 3A)	Intercept	0.717	0.364	*1.971*	0.066
	Parasitoid	-5.538	1.952	*-2.837*	**0.012**
	Species	0.350	0.535	*0.654*	0.522
	Parasitoid:Species	3.084	2.036	*1.515*	0.149
Z. indianus vs *D. hydei* high density (Fig. 3A)	Intercept	-0.005	0.153	*-0.035*	0.973
	Parasitoid	-3.404	0.460	*-7.402*	**< 0.001**
	Species	-1.316	0.242	*-5.445*	**< 0.001**
	Parasitoid:Species	3.388	0.531	*6.380*	**< 0.001**
Z. indianus vs *D. simulans* low density (Fig. 3B)	Intercept	0.209	0.180	*1.159*	0.263
	Parasitoid	-5.029	1.021	*-4.924*	**< 0.001**
	Species	0.736	0.269	*2.737*	**0.015**
	Parasitoid:Species	3.256	1.059	*3.076*	**0.007**
Z. indianus vs *D. simulans* high density (Fig. 3B)	Intercept	-0.317	0.156	*-2.030*	0.059
	Parasitoid	-4.911	1.071	*-4.586*	**0.003**
	Species	0.173	0.220	*0.787*	0.443
	Parasitoid:Species	3.184	1.106	*2.879*	**0.011**
Z. indianus vs. *D. simulans* exposure time (Fig. 4)	Intercept	0.547	0.219	2.499	**0.013**
	Species	1.063	0.358	2.973	**0.003**
	1 hour	-1.933	0.506	-3.819	**< 0.001**
	8 hours	-3.914	1.040	-3.762	**< 0.001**
	24 hours	-3.914	1.040	-3.762	**< 0.001**
	Species:1 hour	1.171	0.704	1.664	0.096
	Species:8 hours	1.899	1.141	1.665	0.096
	Species:24 hours	2.573	1.139	2.258	**0.024**

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