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Integrated Pest Management in California Field Crop Systems:
Management Challenges in Alfalfa and Rice

By

MADISON HENDRICK
DISSERTATION

Submitted in partial satisfaction of the requirements for the degree of

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in

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OFFICE OF GRADUATE STUDIES

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DAVIS

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Committee in Charge

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Table of Contents

DISSERTATION ABSTRACT.....	III
ACKNOWLEDGEMENTS.....	V
CHAPTER 1: WEEVILS WOBBLE, BUT DO THEY FALL? THE STATE OF INSECTICIDE RESISTANCE IN CALIFORNIA POPULATIONS OF ALFALFA WEEVIL (<i>Hypera postica</i> GYLLENHALL).....	1
ABSTRACT.....	1
INTRODUCTION.....	2
MATERIALS AND METHODS.....	5
RESULTS.....	12
DISCUSSION.....	17
WORKS CITED.....	28
TABLES AND FIGURES.....	40
CHAPTER 2: EVALUATING THE DAMAGE POTENTIAL OF TADPOLE SHRIMP (<i>Triops longicaudatus</i> (LeCONTE)) ON CALIFORNIA RICE UNDER DIFFERENT CONDITIONS.....	55
ABSTRACT.....	55
INTRODUCTION.....	56
MATERIALS AND METHODS.....	59
RESULTS.....	64
DISCUSSION.....	67
WORKS CITED.....	75
TABLES AND FIGURES.....	87
CHAPTER 3: ALL SURF, NO TURF: EVALUATING THE MOSQUITOFISH (<i>Gambusia affinis</i>) AS A POTENTIAL BIOLOGICAL CONTROL AGENT FOR TADPOLE SHRIMP (<i>Triops longicaudatus</i> (LeCONTE)) IN CALIFORNIA RICE.....	99
ABSTRACT.....	99
INTRODUCTION.....	100
MATERIALS AND METHODS.....	105
RESULTS.....	109
DISCUSSION.....	114
WORKS CITED.....	123
TABLES AND FIGURES.....	136

Dissertation Abstract

California produces a wide variety of crops, with field crops contributing substantially to the statewide agricultural economy. Many field crops face arthropod pests, and protecting these crops is a constant challenge for growers and pest control advisers. Integrated pest management (IPM) is an ecological approach to pest management that prioritizes non-chemical control measures and uses chemical pesticides as a last resort. However, certain crops, such as alfalfa and rice, are limited in their control options and rely heavily on insecticides for pest management. Though effective, overreliance on these chemicals can lead to insecticide resistance and environmental issues. We explored challenges to integrated pest management to address gaps in current management programs. We examined the scope of pyrethroid resistance in California populations of alfalfa weevil (*Hypera postica*), and used historical pesticide use data to explore management questions. We found both susceptible and resistant populations across the state. We found only weak relationships between historic insecticide usage and modern resistance levels, but identified other trends in management such as changes in application timing and number of applications. In rice, we aimed to examine the damage potential of tadpole shrimp (*Triops longicaudatus*; TPS) under different environmental conditions. The development of both organisms individually is reasonably defined, but their development in tandem and resulting damage potential is not. We found that under increased temperatures, pest pressure from the TPS is increased. Finally, we evaluated mosquitofish (*Gambusia affinis*) as a potential biological control agent for this pest with the goal of adding another control method to growers' toolkit. However, we determined that the mosquitofish is not a suitable control agent for this system. Through these studies, we contributed to improvements in how IPM is approached in field crops. These systems can be challenging given the large

production scale and small margins, but there are still opportunities to make IPM programs more effective and sustainable.

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Weevils wobble, but do they fall? The state of insecticide resistance in California populations of alfalfa weevil (*Hypera postica* Gyllenhal)

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Key words: Integrated Pest Management, Insecticide Resistance, Alfalfa Weevil, Pesticide Use

ABSTRACT

The alfalfa weevil (*Hypera postica* Gyllenhal) is the key pest of California alfalfa, and weevil management is largely limited to insecticides. Additionally, few insecticides are registered for use against alfalfa weevil, severely restricting options for management and increasing the chances for insecticide resistance. In the late 2010s, resistance to lambda-cyhalothrin was confirmed in isolated areas of California. A critical step in managing resistance is monitoring resistance levels across to understand the severity of the issue and preserve the limited control options available. We collected weevils from various populations across California and performed dose-response lab assays using vials coated with lambda-cyhalothrin. We found many susceptible populations, with isolated instances of resistance. We also attempted to link historic insecticide use data from the Department of Pesticide Regulation's pesticide use database to identify influential factors in resistance development. We found weak relationships between past insecticide use and modern resistance, but our resistance data set was limited. We also used the pesticide use data to explore various trends in insecticide usage and found that in some parts of the state, lambda-cyhalothrin applications are trending earlier and that early applications increase the likelihood of secondary sprays. This project aimed to address management challenges in alfalfa by highlighting ways in which insecticides are being used in this system so management can be regionally tailored and our current management tools can be preserved.

Introduction:

Chemical insecticides revolutionized pest management, but their widespread use has been accompanied by the evolution of insecticide resistance in over 500 arthropod pest species, reducing efficacy and increasing management costs (Forgash, 1984; Sparks et al., 2021; Tabashnik et al., 2014). Integrated pest management (IPM) in its current paradigm recommends alternative strategies, such as cultural or biological control, as the first steps in pest management, and using insecticides judiciously or as a last resort. Insecticide resistance management (IRM) strategies slow resistance development and maintain chemical efficacy.

One key IRM strategy is chemical rotation of multiple modes of action (MoA). By rotating or alternating MoAs, the selection pressure is removed from a particular target site for a time, reducing the risk of mutations that confer resistance to that chemistry. The success of MoA rotation hinges on the diversity of available insecticide chemistries and non-chemical options that can provide management support. When pests develop insecticide resistance, growers are often forced to cycle through remaining, effective MoAs until a new chemistry or strategy is made available. This is a challenge that has been documented across a variety of pest and crop systems. For example, diamondback moth (*Plutella xylostella*) was one of the first pests to develop resistance to DDT in the 1950s and has since developed resistance to more than 80 chemistries (Philips et al., 2014). Colorado potato beetle (*Leptinotarsa decimlineata*) is another infamous pest that rapidly develops resistance to even novel methods such as RNAi formulations, driving the need for continued innovation in insecticide development (Chen et al., 2023). In such cases, the constant selective pressures exerted on the pests through overuse or reliance on a particular chemistry can remove that option from the available list of insecticides, reducing the number of options growers have for management. This can force reliance on a new

chemistry, and this cycle can accelerate resistance development to the point of outpacing product development.

The introduction of invasive species further complicates management practices, as the lack of established monitoring tools or control methods forces growers to rely on chemicals without adequate information about their efficacy, or to simply endure economic losses if research on chemical or alternative control strategies for a given pest are lacking. Tomato pinworm (*Tuta absoluta*), for example, has developed resistance to multiple MoAs and resistant phenotypes have been introduced into new areas. In the case of its Mediterranean introduction, growers were forced to rely upon organophosphates and pyrethroids due to a lack of registered options, which ultimately proved ineffective for management (Guedes et al., 2019). The overreliance on chemical control and the resulting insecticide resistance has shown the need for biological control in this system. Many species have been identified and even imported into South America as biocontrol agents, but there remains a need for more rigorous studies to develop effective biological control programs (Salas Gervassio et al., 2019). These cases underscore that, even within the IPM framework, a lack of alternative options maintains the reliance on insecticides. Though the current paradigm of integrated pest management (IPM) holds chemical control as a “last resort” after the implementation of other means of pest control, chemical insecticides remain the most effective or only effective means of pest management in some cropping systems (Sparks & Nauen, 2015).

In California, alfalfa is one such crop with limited control options for controlling its key pest, the alfalfa weevil (*Hypera postica* Gyllenhal). Alfalfa is a perennial crop that is harvested multiple times in a year, with the initial harvest or cutting being the most valuable economically (Putnam 2021). The alfalfa weevil is an invasive, early season pest whose larval stage feeds

heavily on the first cutting of alfalfa for two to four weeks before pupating (Pellissier et al., 2017). The feeding damage heavily defoliates and skeletonizes the plants, reducing the overall yield and quality (Long et al., 2017.; Pellissier et al., 2017). Growers in California depend on chemical insecticides for alfalfa weevil control, as biological control agent populations are not robust enough and do not exist evenly across the state to effectively manage weevil populations and cultural practices such as early harvest can sacrifice yield and leave weevils in the field (Long et al., 2017). Chemical control options are also limited. Relatively few chemicals are registered for use in alfalfa for alfalfa weevil, and those commonly used are: lambda-cyhalothrin (IRAC Group 3A), indoxacarb (IRAC Group 22A), beta-cyfluthrin (IRAC Group 3A), zeta-cypermethrin (IRAC Group 3A), malathion (IRAC Group 1B), spinosad (IRAC Group 5) (Godfrey et al., 2024). Following the development of resistance to several organophosphates in the past, growers now typically rely on pyrethroids (Rodbell et al., 2024). This newfound dominance of pyrethroids leaves growers vulnerable again to the cycle of insecticide resistance.

Though pyrethroids are typically effective, some populations of alfalfa weevil across the western United States have developed resistance to lambda-cyhalothrin, with cross resistance found to zeta-cypermethrin (Rodbell et al., 2022, 2024). Within California, there have been reports in the 2010s of field control failures in the Palo Verde Valley in the south and in Scott's Valley of northern California. These control failures were further validated by lab assays (Orloff and Godfrey, *unpublished data* 2016). Growers in the San Joaquin Valley have also, anecdotally, reported control failures, suggesting that resistance may be more widespread than previously documented. The overall level of pyrethroid resistance in the state of California is not well defined and, even within tested areas, there could be a range of susceptibility to pyrethroids. Standardized assays such as internally coated vial assays are useful for assessing levels of

resistance within pest populations and can provide early warnings for chemical control strategies (Miller et al., 2010). Because there are so few control methods for this pest, understanding how well the current options work is a necessary first step in resistance management. Continued overuse of pyrethroids can increase the risk of resistance development, and may result in a shift or overreliance on indoxacarb (Rodbell et al., 2024).

We aimed to address pyrethroid resistance in California populations of alfalfa weevil and the factors that may be influencing its development. The three primary objectives were to 1) survey lambda-cyhalothrin resistance levels in alfalfa weevil populations across California, 2) use the California Department of Pesticide Regulation pesticide use report (PUR) database with our LC_{50} s to understand how historical pesticide use influenced resistance today and 3) use the PUR database for exploratory analyses to identify trends and changes in insecticide use over the last 30 years.

Materials and Methods:

Objective 1:

Field Sites:

Weevils were collected from commercial alfalfa fields throughout California. We collected from a total of 34 fields across 10 counties. Some sites were targeted based on previous research showing resistance or following reports of field control failure. Other fields were chosen without prior knowledge of resistance status. The state was divided into four geographic regions by county for sampling and analyses: southern CA, San Joaquin Valley (SJV), Sacramento Valley (SacV), and northern CA. Regions were defined loosely on weevil phenology and geographic location. Field sites were at least one mile apart, with the exception of two field sites

in the southern region due to sampling availability. All locations, regions, and the associated counties can be found in [Table 2](#).

Bioassays:

We used a sweep net to collect weevils from the field locations, which were sampled at various points along the edges and in the center of the field. Weevils were then transferred into a 5 gallon bucket with plant material with paint straining bags placed over top to ensure airflow. Extra weevils were collected and placed into paper bags with plant material and then transported in a 40qt Coleman PowerChill portable plug in-cooler to ensure population survivorship after transport. Once in the lab, weevils populations were divided into multiple one-gallon ice cream buckets with plant material and a mesh top to prevent overcrowding. The weevils rested and fed overnight to acclimate to lab conditions and reduce background mortality.

To test for resistance, we performed internally coated vial assays. We gently sifted weevils from the plant matter using a Fisherbrand No. 5 (4.00mm) sieve. We selected second and third instar larvae for the assay to avoid pupation. Weevils were placed 10 to a vial. Each assay tested a range of 8 doses, including an untreated control. Doses can be found in Table 1. Doses were chosen based off a serial dilution of the technical grade AI with the base dose being 1.92 fl oz, the upper end of the label rate. We ran five replicates of each dose. We placed 1ml of the insecticide solution into 40ml vials, then rolled on a hot dog roller with no heat until dry. Control vials were treated with 1ml of acetone. This project was a collaboration with Montana State University, and we conducted the assays in accordance with their established methodology (Rodbell et al. 2022).

After weevils were placed into the vials, a square of mesh fabric was placed over the opening. The lids, modified with holes and covered with metal mesh to prevent escape, were

screwed on over the mesh fabric. The interior mesh served to keep weevils from gathering in the untreated lid and avoiding the insecticide-coated portions of the vial. The vials were placed randomly in a rack and set horizontally on the lab bench to allow better movement for the weevils. They were kept in a dark room at room temperature for 24 hours before evaluation.

We evaluated the assays by dumping the weevils into a petri dish 15cm above a low heat on a hot plate. Alfalfa weevil larvae have a defensive curl mechanism and were sluggish after assaying. The heat provided enough stimulation for them to uncurl and attempt to move. Weevils that could walk at least one full body length of distance without impairment were classified as alive, and any signs of impairment or outright dead weevils were classified as moribund for analysis.

We fit the mortality data to a two-parameter log-logistic function using the drc package (Ritz et al., 2015) in R version 2022.12.0+353. Prior to analysis, we used Abbott's formula for mortality correction on all populations with control mortality higher than 0 but less than 20%. Populations with control mortality higher than 20% were discarded. Separate dose-response curves were generated for each population to generate population-specific LC_{50} values. All models were generated with lower and upper limits fixed at 0 and 1 respectively, while slope and intercept, the LC_{50} value, were not fixed.

Objective 2:

Pesticide use data:

California's Department of Pesticide Regulation has created the Pesticide Use Report (PUR) database, which has records of every pesticide application made in the state since 1990. We used the PUR database to analyze the relationship between historic pesticide usage and our

LC₅₀ data. We first had to prepare the PUR data for use. The PUR database contained records of pesticide application from 1990 to 2023. The database reports usage at the field-level spatial scale. Each field falls within a 1mi² area in a grid system developed by the Bureau of Land Management. Each square is labeled by county, meridian, township, region, and section (COMTRS). We first checked extracted data for clear errors to remove them prior to analyses. Entries that were clear mistakes, such as a single COMTRS totaling over 640 acres or over 350 pounds of a given material being applied, were removed from the dataset.

For Objective 2, we summarized the data at the COMTRS level. From there, we were able to calculate the acreage of alfalfa planted on a year-to-year basis by combining data from all insecticide, herbicide, and fungicide applications and using the maximum acreages planted in each COMTRS as an “acres planted” value. This value was attached to each COMTRS in its own column. We then created a “spray proportion” value using the “acres treated” input for a given chemical divided by the acres planted. This gave us the proportion of a COMTRS that was treated with a given insecticide. This step was crucial, as it allowed us to compare use patterns across chemicals with different application rates. The proportion of acres treated to planted was generated for every insecticide application in the dataset.

We focused on a subset of the insecticide use data from the weevil seasons, which varied by region in California, because weevils are primarily a pest of the first cutting and are only active as larvae in the field for about one month in a given field. Insecticide applications of the focal active ingredients made during this period are likely either targeting weevils and/or would be relevant for selection pressure and thus insecticide resistance development. We designated this time period to encompass the onset and the end of weevil larvae activity for the first generation in alfalfa. In the Southern region, the season extended from February to April. In the

SJV and SacV, the season extended from February through May. These regions occupy the southern and northern parts of the Central Valley respectively and are geographically similar. The Northern weevil season extended from April to June.

We attached the location data of our LC₅₀s to the corresponding COMTRS unit. We used this to “link” our resistance and insecticide usage datasets. Alfalfa weevils have a very slow dispersal rate of about 3 miles a year and tend to stay within the confines of the field when present. However, we assumed that activity in neighboring fields such as insecticide run-off or drift, or other growing practices may influence the target field. To account for this, after identifying the COMTRS that corresponded to a collection site, we found the COMTRS immediately adjacent on all sides and corners. This effectively created a 3x3 grid in which our collection points sat in the middle. We used the total summation of acres treated and acres planted across all COMTRS in this grid to get the spray proportion for the grid. This value was attributed to the COMTRS containing our collection points and used in the analyses. Since our field collections began in 2020 and continued until 2023, and collection dates varied by year within regions. To simplify analysis, we used historical data up until 2019. We then subset the overall dataset by both chemicals of interest and time. To subset by chemical, we partitioned use by four of the most used pyrethroids (lambda-cyhalothrin, zeta-cypermethrin, beta-cyfluthrin, and alpha-cypermethrin) and then by using only lambda-cyhalothrin as the source for the proportion of acres treated. From these datasets, we subset again to analyze whether specific periods in time had a particular effect on LC₅₀. The time designations were: 1990 to 2019 (whole dataset), 2010 to 2019 (pyrethroid adaptation in management), and 2015 to 2019 (recent years). We created datasets for analyses for all combinations of data for a total of 6 analyses. All datasets were analyzed using the same linear model: $\log_{10}(\text{LC}_{50})$ as a response to the interaction

of fixed effects proportion of acres treated and region. Model fit was assessed using adjusted R^2 values. Predictor significance was evaluated using analysis of variance (ANOVA).

Objective 3:

We also used the PUR database to answer some exploratory questions. Objective 3 had three primary sub-objectives to explore insecticide use in alfalfa: a) how use of certain insecticides has changed over time; b) how many insecticide applications are made within a season; c) how application timing has changed through time. Sub-objectives b) and c) led to a fourth, related question: d) is there a relationship between the initial insecticide application timing and how many applications are made in a season? Prior to any analyses the PUR data was prepared and cleaned as described in Objective 2.

To assess how insecticide use has changed over time (3a), we used the same calculation method of acreage treated to acreage planted to determine the proportion of acreage per COMTRS that received an application of a given insecticide. In this case, we were interested in the four most-used pyrethroids (lambda-cyhalothrin, beta-cyfluthrin, alpha-cypermethrin, and zeta-cypermethrin) and indoxacarb (IRAC Group 22). Acreage was summed by COMTRS and then county, to get the total proportion treated annually.

We then examined insecticide application counts within a growing season (3b). Growers typically only need to treat their field once in a season to achieve weevil control. However, multiple applications may be made, for instance, if they are experiencing field control failures. To address this, we examined lambda-cyhalothrin, indoxacarb, and the four most-used pyrethroids separately. Data was grouped at the field level according to each chemical, and then the number of applications were counted, and the fields were categorized accordingly. Fields

were denoted as receiving either 0, 1, 2, or 3+ applications. We used these categories to calculate the proportion of fields receiving applications out of the total number of fields, then grouped by region.

We then examined application timing across years, potentially indicating changes in grower behavior for applications or shifts in phenology (3c). To assess changes in application timing, we subset by the same weevil seasons as indicated above and focused solely on lambda-cyhalothrin because it is the most widely used and weevils have developed resistance to it. We then used the *rq* function in quantreg package (Koenker, 2025) to perform quantile regression of day of year in response to year at the 20th, 50th, and 80th percentiles ($\tau = 0.2, 0.5, 0.8$). Early applications constituted, the first 20% of applications, late applications would constitute the last 20% of applications, and the middle 60% of applications comprised the “typical” treatment range. We used the *rq* function in the quantreg R package. We modeled regions individually, using one model per τ value, with day of year as a response to year.

Finally, we explored the relationship between application timing and the number of applications made in a season (3d). We grouped our dataset by region and mean-centered the first application date to account for our regional differences in timing. The mean-centered date served as a benchmark for all other applications. We then grouped each field and its associated grower ID and found the first application date for each field. We also found the 20th percentile application date for each region, each year. Meaning, we established a threshold for early applications by defining the upper limit of “early” applications in the region. If a field’s first application date fell within that 20%, it was deemed an early application. Next, we counted how many total applications each field received in a growing season each year. If a field was sprayed once, its spray frequency was denoted as “1” and “2+” if it received more than one application to

create a binary response variable for modeling. From here, we used generalized linear models for each region to perform binomial logistic regression to show the relationship between spray frequency as a response to the interaction of the fixed effects first day of application and year. We then used the *emmeans* package (Lenth 2023) in R to find the estimated marginal means to get the probability of secondary sprays in result to spray timing. We used the *emtrends* function to extract the year-specific slopes of the effect of first day of application timing, and the *test* function to statistically identify years in which this effect was significant.

Results:

Bioassay:

A wide range of susceptibility was detected through our bioassays. Detailed bioassay results and LC₅₀s can be found in [Table 2](#). A total of 34 populations across 9 counties were sampled. Six populations were sampled in the Southern region, 13 in the San Joaquin Valley, seven in the Sacramento Valley, and eight in the Northern region. Susceptibilities ranged from 0.001 µg/cm² to 119.929 µg/cm². The most resistant population was found in Riverside County (southern region). Resistance varied greatly within Riverside. Of the three populations sampled, two populations came from separate fields within the same COMTRS unit, one of which was highly susceptible (0.006 µg/cm²) and one of which was highly resistant (119.929 µg/cm²). The third population in that county was also highly resistant, with an LC₅₀ of 1.458 µg/cm². One resistant population was found in Yolo County, with an LC₅₀ of 1.057 µg/cm². Moderately resistant populations were found in Kings County (San Joaquin Valley). Two of the four populations had LC₅₀s of 0.405 µg/cm² and 0.631 µg/cm², with the other populations registering as highly susceptible at 0.007 µg/cm² and 0.001 µg/cm².

DPR PUR Analysis:

We found a weak relationship between the proportion of acres treated and region. Model statistics can be found in [Table 3](#). For all years of historical usage for the four most-used pyrethroids, we found that the interaction between the proportion of acres treated and region had a marginally significant effect ($p = 0.053$) on LC₅₀ values. Similarly, for lambda-cyhalothrin and the same time period, the interaction was again almost significant ($p = 0.054$). When including only years 2010 through 2019, the interaction between the proportion of acres treated and region was not significant when looking at either all four common pyrethroids or just lambda-cyhalothrin ($p = 0.417$ and $p = 0.356$, respectively). Region was significant when examining all four pyrethroids ($p = 0.033$), and both proportion of acres treated and region independently ($p = 0.040$ and $p = 0.045$, respectively) were significant when looking at only lambda-cyhalothrin. At our most restrictive time interval of 2015 through 2019, the interaction was again, not significant ($p = 0.660$ and $p = 0.422$, respectively). When looking at all four pyrethroids, region was again significant ($p = 0.023$). When looking at lambda-cyhalothrin only, the proportion of acres treated was marginally significant, and region was significant ($p = 0.081$ and $p = 0.037$, respectively).

We ran the same models, excluding outlier points, and found that the interaction in the full time scale, lambda-cyhalothrin model, the interaction was significant ($p = 0.006$), but only region was significant when examining all four pyrethroids ($p = 0.002$). When looking at the 2010 to 2019 period, the results did not change. When looking at the 2015 to 2019 period, the results did not change. However, the proportion acres treated factor was significant in the lambda-cyhalothrin only analysis ($p = 0.014$). We removed a total of three outliers from our LC₅₀

dataset for additional analysis, concerned that they may be skewing the data. Removing those points made only region a significant factor in the full model (all years, all pyrethroids) and made the interaction between acres treated and region significant in the 1990 to 2019 and 2015 to 2019 time periods when examining only lambda-cyhalothrin.

Objective 3:

Historic Insecticide Use Trends:

Insecticide usage trends over time can be found in [Figure 1](#). Proportion of acreage treated versus what was planted was used as a metric for use to account for different chemistries having different field application rates. The chemicals included in this were the four commonly used pyrethroids (lambda-cyhalothrin, beta-cyfluthrin, alpha-cypermethrin, and zeta-cypermethrin) and indoxacarb (IRAC Group 22). Other chemicals such as chlorpyrifos, malathion, and spinosad are or have been registered for use in California alfalfa, but were not included in this chart. Chlorpyrifos, though commonly used, has slowly been replaced by pyrethroids and was ultimately removed from the registered products list in 2019.

By plotting insecticide usage over time, we identified several trends in usage across all regions. Madera and Merced Counties in the SJV region have both seen a rapid decline in the amount of lambda-cyhalothrin applied since the late 2010s. Growers in these areas have, anecdotally, reported field control failures and possible resistance to lambda-cyhalothrin. In contrast, we saw a stark increase in the acreage being treated with indoxacarb. This switch was likely in response to developing insecticide resistance. Similarly, in Imperial and Riverside Counties where we know resistance has been reported and verified (Orloff, Godfrey), there was a decline in lambda usage met with an increase of indoxacarb usage. At this point in time, both

chemicals are used in almost equal amounts in each county. Siskiyou in the Northern region, where Scott Valley is located and where resistance was severe in 2018, has historically used beta-cyfluthrin in favor over lambda-cyhalothrin, but usage of both chemicals dropped steeply in the 2010s. Use of lambda-cyhalothrin has since rebounded, but indoxacarb usage is nearly equal in terms of proportion of acres treated.

Changes in Application Dates:

All regions showed changes in application timing at all percentiles. Model statistics can be found in *Table 4*, and all models except two showed significant change in application dates over time ($p < 0.001$). We found a shift forward in the 20th percentile for all regions except Northern. The Southern region saw a forward shift of 0.60 days, the San Joaquin Valley saw 0.20 days, and the Sacramento Valley saw 0.47 days per year. The Northern region's early applications shifted back by 1.90 days per year. The Sacramento Valley and Southern regions saw a shift forward in median application date by 0.14 and 0.50 days per year respectively. Conversely, the Northern and San Joaquin Valley regions saw median applications shift back in time by 1.22 and 0.08 days respectively. Finally, all regions except Southern saw the 80th percentile trend later in the year (0.50, 0.35, 0.32 days per year respectively). The Southern region did not experience a change in its 80th percentile, and year was not significant in that model ($p = 1.00$).

Number of Applications:

We summarized the historic pesticide use data within our designated regional weevil seasons to count the number of applications a single field received within one season. Line graphs detailing the proportion of fields treated once, twice, more than twice, or not at all can be found in [Figure 3](#). The SJV region experienced 3 points in which the proportion of fields

receiving no treatment and fields receiving 1 treatment with lambda-cyhalothrin switched to favor one over the other. Usage also appeared to be in a sort of equilibrium, in which from the late 2000s onwards, approximately 50% of fields were treated only once. From 2010 onwards, there is a steady increase in fields treated once with Indoxacarb. In the SacV region, most fields are treated once with lambda-cyhalothrin during the season. In the late 2010s, there is an uptick in fields receiving 2 applications. Around this same time period, the region also saw an increase in indoxacarb usage. The Southern region also saw a spike in fields receiving 2 applications during the 2010s, which coincides with an overall increase in the proportion of fields receiving at least one indoxacarb application.

Number of Sprays in Response to Application Timing:

In all regions except northern, the interaction between first day of application and year was significant (Northern $\chi^2 = 0.48$, SacV, SJV, Southern $\chi^2 < 0.001$). Full model statistics can be found in

Table 5. Meaning, growers in those regions that made earlier applications were likely to make a secondary application. However, the effect of the first day of application was not significant at all years, and the slope of the predicted probability was not negative in all years across all regions. Year specific slopes and p values can be found in *Table 6*. In the northern region, only 2 years were identified in which this effect was significant. In the Sacramento Valley, every year since 2009 excluding 2011, 2012, 2017, and 2021 were significant. In the San Joaquin Valley, in all significant years, every year except 2015 had a negative slope. In 2015, the slope was positive, meaning the predicted probability of secondary sprays was actually higher in the latter half of the season. In the southern region, all years since 2000 excluding 2001 and 2002

were significant, with the probabilities of secondary sprays ranging from about 50% at its lowest to nearly 100% at their highest (*Figure 7*).

Discussion:

This study aimed to elucidate pyrethroid resistance in alfalfa weevil across California, as well as trends in insecticide usage relevant to resistance development and overall weevil management strategies and how it may affect resistance. Through our sampling efforts found that resistance was variable across the state, and seemingly localized at small spatial scales within counties. Through investigation of insecticide usage trends, we found that there is a relationship between early applications and an increased likelihood of secondary sprays. As of now, the consequences of these earlier applications are unknown in terms of resistance management.

We found resistance to be highly variable among different populations and seemingly isolated to small areas within counties. This study was part of a larger study by Rodbell et al. at Montana State University, who also identified resistance in other western states. Their study found that other states struggling with pyrethroid resistant alfalfa weevil seemed to have issues in isolated areas, and so noted the localized nature of resistance in this system (Rodbell et al., 2022, 2024). We found variability in resistance at small spatial scales within counties, reflecting what was found in other western states. Many of the populations were found to be susceptible, but resistant populations were found in all regions except the Northern region. We found two highly resistant populations out of three collected from Riverside County, in our Southern region, the least resistant of which having an LC^{50} value over 4 times the highest recommended field dosage. The few moderately resistant populations found in Kings and Yolo Counties all had LC^{50}

values between 2 and 3 times the highest recommended field dosage. We expected to find at least one resistant population in the northern region, as the original reports of pyrethroid resistance in California came from the Intermountain region up north (Orloff and Godfrey; *unpublished data*). However, all sampled populations from two counties in the Northern region registered as susceptible after testing. Changes in management practices after the confirmation of resistance or potential fitness costs associated with resistance may explain why resistance was not found in this area. Additionally, our sampling efforts were not comprehensive and it is possible that we simply did not sample a population that was still resistant.

Understanding the geographic range of resistance is crucial for management, as it can allow for targeted tactics, but there needs to be a consideration for the local landscape, management history, and current resistance risk level. Multiple strains of this pest are found throughout the entirety of the state across varying climate zones, but their phenology is impacted by climate diversity, which affects rates of eclosion, larval and adult activity in the fields (Long et al., n.d.; Stilwell et al., 2010). Ensuring a baseline understanding of the pest's phenology in a given area could be important, especially as climate changes, rather than applying a "one size fits all" approach to management. Alfalfa weevil is also a species with a relatively low dispersal rate, at an estimated range of up to three miles per year, minimizing gene flow between populations (Pellissier et al., 2017). In contrast to the relatively sedentary alfalfa weevil, the fall armyworm (*Spodoptera frugiperda*) is a highly mobile pest. However, evidence suggests that local pest management matters more in terms of resistance, as the movement of resistant populations into susceptible areas did not necessarily mean an increase in resistance into the new area. Instead, local pest management had a stronger effect on local LC₅₀s (Arias et al., 2019). The relative isolation of certain production areas or the intensity of production areas may influence the

severity of pyrethroid resistance by further limiting gene flow from susceptible individuals or exposing populations to higher-than-average selective pressures (Rodbell et al., 2022).

Susceptible gene reservoirs are a strategy used in resistance management (Georghiou and Taylor, 1977), and investigation of the local landscape in areas of pyrethroid resistant alfalfa weevil may provide insight for maintaining susceptibility in the population. In green peach aphid (*Myzus persicae*), the frequency of resistance alleles in orchard aphid populations and found that weeds surrounding the fields acted as a reservoir for susceptible alleles whereas the orchards maintained the resistant alleles (Rubio-Melendez et al., 2017). Knowing if and where those areas exist, preserving them, and promoting gene flow in and out of the field could be a useful tool for managing weevil resistance. Regional surveys using dose-response curves to generate LC_{50} values for populations, such as ours, are among the common strategies to assess resistance, but there is an acknowledged need for improvement in scale and scope of said surveys to enhance decision making (Guedes, 2017). The localized nature of weevil resistance thus far suggests that local management efforts have a strong impact on pyrethroid resistance. Tailoring management plans, particularly in areas where resistance has not yet developed will be crucial for prolonging the availability of effective chemicals.

Through our analyses, we found that there is a relationship between LC_{50} , historical usage and region, though it depends on the years examined. When looking at all four, commonly used pyrethroids, the interaction between the proportion of acres treated and region was only a slightly significant predictor in the model examining the full 30 years of data, meaning that the effect of the proportion of acres treated on LC_{50} may have varied by region. Across all common pyrethroid models, the proportion of acres treated was at best only marginally significant, and the inclusion of multiple chemistries likely supplied too much noise to the data to draw clear

conclusions about acreage's effect on LC_{50} . Conversely, when the model was run using data across all years for only lambda-cyhalothrin applications, both proportion of acres treated and region were independently significant predictors. The interaction of these two factors was marginally significant across all years of data, again indicating a weak relationship between them and their effect on LC_{50} . When examining lambda-cyhalothrin usage during the 2010 to 2019 time period, there was again a weak relationship between LC_{50} and the interaction of the two predictors, but both predictors were significant independently. The only time delineation in which the proportion of acres treated was not significant was 2015 to 2019, likely due to less application data being included in the model to begin with. In all models, regardless of chemical inclusion or time frame, region was significant. Region being significant in all models implies that differences in LC_{50} are more or less a result of regional differences, which was observable prior to data analysis.

Our dataset and model did have some limitations. Our model is somewhat data limited, with 34 sampling locations total. The first 2 years of the project were heavily impacted by the COVID-19 pandemic, which severely limited weevil sampling capabilities. Thus, the overall spread of our location data is rather limited and lacking in certain areas. Our model is also limited in the factors that were used in the analysis. Future studies in this system or others may want to investigate additional factors such as number of applications over time or on average, the evenness of applications across time, or specific grower behaviors not identified in this study. Overall, while our models do show some relationship between historical pesticide usage and current day resistance data, the relationship is not strong enough to make significant predictions of resistance levels statewide.

While there was not a strong relationship between measured resistance levels and historic insecticide usage in fields near the collection point, we do see patterns in usage statewide and at the county level that reflect changes in chemical usage. Most counties relevant to this project have relied heavily on lambda-cyhalothrin. In recent years, some areas have been making the switch to indoxacarb either as a response to or a preventative measure for insecticide resistance. Riverside County illustrates a responsive switch to indoxacarb. Indoxacarb usage in this county rose from under 20% to 50% between 2017 and 2019. Conversely, lambda-cyhalothrin usage followed the reverse trajectory from 2019 to 2021 and usage dropped from 50% of fields being treated to fewer than 20%. This change in usage coincides with years of confirmed insecticide resistance in the area, and these usage trends shown in [Figure 1](#) are indicative of the growers' direct response to this issue. Similar trends in which lambda-cyhalothrin and indoxacarb usage is almost entirely inverted over the span of a few years occurred in Madera and Merced Counties, an area where resistance was suspected but not confirmed. Though we can't say with certainty what the drivers of those changes in usage are, it is very likely that growers in that area are making a preventative switch to a new MoA to slow or prevent the further development of resistance.

Other Type II pyrethroids similar to lambda-cyhalothrin are used in management programs to a lesser extent but still contribute to overall pyrethroid exposure and selection pressure. Usage of these chemicals statewide remains fairly low, with the exception of the Northern region, where beta-cyfluthrin usage was almost comparable to lambda-cyhalothrin usage. In Siskiyou County, beta-cyfluthrin was favored over lambda-cyhalothrin until 2017, and both chemicals saw a steep decline in usage in the late 2010s. This decline in use coincides with both an increase in indoxacarb usage and with noted control failures in the field and a period of

confirmed resistance in the region (Rodbell & Wanner, 2021; Orloff, 2016; *unpublished data*). Notably, in Imperial, Riverside, Madera, Merced, and Siskiyou Counties, where pyrethroid resistance was either confirmed or suspected, use of other Type II pyrethroids tended to decline when lambda-cyhalothrin use declines. Though these other Type II pyrethroids are not typically used as often as lambda-cyhalothrin, their efficacy still stands to suffer from the development of resistance. Weevil populations resistant to lambda-cyhalothrin have been found to be cross-resistant to other Type II pyrethroids, and mortality from beta-cyfluthrin was strongly correlated with mortality from lambda-cyhalothrin, meaning it may be the least effective chemical against resistant weevil populations (Rodbell et al., 2024). Again, though we cannot confirm the drivers behind these changes in usage, it is reasonable given the evidence for cross-resistance to assume that the decline in usage is tied to a decline in efficacy. The reduced efficacy of lambda-cyhalothrin has demonstrably resulted in the loss of additional pyrethroids, further limiting the suite of effective chemistries in this system.

Across all regions, fields were typically only treated once during a season, but there were several notable spikes in secondary applications. In the southern region during the mid 2010s, there was a notable increase of fields treated twice with lambda-cyhalothrin during the season, the peak aligning with when resistance issues were most. These repeated applications may have been both a sign of resistance development and how resistance development accelerated. Growers experiencing field control failures may switch to a different chemical, or other methods when available, as means of adaptive management (Rosenheim, 2021). During this period, the region's use of indoxacarb had also peaked during the period of resistance issues, as growers may have been switching chemicals as a response to resistance. Resistance management guidelines encourage the rotation of effective chemistries to moderate the selection pressure

exerted by a single chemical (Helps et al., 2020; Sparks & Nauen, 2015), but the switch to indoxacarb may have come too late for resistance prevention. It appears from our data as though growers applied lambda-cyhalothrin and then followed it with a second application of lambda-cyhalothrin later in the season. While multiple applications of the same chemistry can certainly accelerate resistance, using indoxacarb as the secondary spray would be better for moderating resistance pressure. There is also evidence to suggest that, particularly in the case of sexually reproducing insects like alfalfa weevil, insecticide mixtures at either label or reduced-rate applications are more effective for controlling pests while managing resistance development (Helps et al., 2020).

Ensuring high levels of control from a single application and keeping applications to a minimum in a season is crucial for resistance management. Multiple factors can affect the level of exposure an insect actually receives, such as chemical degradation to rain, sun or other climate factors, improper or spotty application, or insect movement and behavior within the field (Desneux et al., 2007). Within populations exposed to lethal insecticide doses, some individuals may only receive sublethal exposure, which can affect subsequent generations. Sublethal insecticide exposure in Colorado potato beetle (*Leptinotarsa decimlineata*) had negative effects within a generation, but subsequent generations benefited from parental exposure to insecticides (Margus et al., 2024). The initial exposure resulted in negative consequences such as a reduced egg-hatch rate, but the subsequent generations had higher fitness and survivability and higher body mass (Margus et al., 2024). Even if weevils are only being exposed to insecticides once in a generation, this single exposure is still exerting selective pressures that may benefit the next generation and increase the likelihood of resistance development.

We also identified changes in application timing across the state and all four regions have had an appreciable shift in application timing. All regions except for the northern region did see a gradual increase in early applications. This means that the earliest 20% of applications are getting earlier each year. In the southern region and Sacramento Valley, we also saw a shift forward for median application dates, meaning that the middle 60% of applications also appear to be shifting forward in time. The Sacramento and San Joaquin Valleys also saw the last 20% of applications move backwards in time. Interestingly, the forward shift in early applications in the Sacramento Valley, San Joaquin Valley, and Southern region seems to imply that the early period of alfalfa growth is extending, which could impact management if the period of weevil presence is also extending. Changes in pest phenology as a result of climate change, including earlier emergence and extended periods of activity by early season pests, has been noted (Forrest, 2016). Though it is unclear now if early applications are being made in response to pest presence or prophylactically, further investigation of weevil phenology may be necessary for management. In the case of the Sacramento and San Joaquin Valleys, early applications have moved forward by less than two weeks across the span of 30 years. The median application date has barely changed at all. In these regions, changes in application timing are likely negligible. The shift forward in time is much more pronounced in the Southern region, and these changes are more likely to have a stronger impact on management practices moving forward.

One unintended consequence of early sprays may be the need for additional sprays later in the season. We found that in all four regions, the interaction between the first day of application of our four target pyrethroids and year shows that early applications increased the probability of secondary applications. Growers, historically, tend to only treat their fields once during a season, but secondary applications have been more common in recent years. Within the

same time frame, applications have shifted earlier in the season, raising the question of whether earlier applications increase the likelihood of additional applications later in the season. In all regions except for the Southern, the likelihood of secondary sprays drops significantly at the mean day, or middle of the growing season. This was especially true of the southern region, in which the effect of application date was significant in each year. From a resistance management standpoint, this implies that early sprays may result in adding additional selection pressure later into the season. Early applications do not guarantee a secondary spray, but the likelihood that a field receives a second spray increases significantly. This effect may be due in part to how we defined the weevil season. For example, a spray made within the first month of the season leaves up to three months of time in which a second application may be necessary. A spray made in the last month may be so close to harvest there is no time for a second application. It is important to acknowledge that the percentage of fields receiving secondary applications is very low. These early sprays may not be inherently harmful, but in areas where resistance is actively developing or is a concern, growers should follow IPM and IRM guidelines and delay sprays until it is economically advantageous to do so. Precautionary, early pyrethroid applications may be unnecessary as defined by economic thresholds; thus, they may be contributing to pyrethroid resistance development (McClure et al., 2023).

The exact drivers of these earlier applications are not yet known, and their long-term effects are not well understood. Climate change is of great concern for California agriculture, as increasing temperatures and climate instability could lead to altered synchrony in pest and plant phenology, increased pest pressure, and changes in management tactics and efficacy (Delcour et al., 2015; Sharma, 2014; Skendžić et al., 2021). Rising temperatures raises the concern of additional generations in some pests, but a study performed in Spain posits that no more than two

generations of alfalfa weevil would be possible in a year up to 28C, after which reproductive capacity suffers (Levi-Mourao et al. 2022). The issue, then, would be with the annual emergence of weevils in the alfalfa fields. In southern regions of the United States where winters are already mild, including southern California, alfalfa weevils have greater variability in oviposition periods and may lay more eggs during winter months than alfalfa weevils in colder climates (Stilwell et al., 2010). Increased temperatures could lead to more winter oviposition across the state, resulting in larger spring hatches. In addition to changes to a species' voltinism, climate change has been linked to other phenological changes such as early emergence and delayed pupation (Bale & Hayward, 2010; Forrest, 2016; Singer & Parmesan, 2010). If weevils begin emerging early and emergence is not uniform, or if emerging populations are larger, not only could we see earlier applications, but we could see multiple applications. Early or staggered emergence may also result in more damage to the crop via feeding on the alfalfa before it reaches a more tolerant growth stage, thus incurring more damage if unmitigated (Stilwell et al., 2010). Growers responding to early, uneven, or extended periods of alfalfa weevil could increase the amount of selective pressure the insects are exposed to and increases the risk of insecticide resistance. Furthermore, it has been demonstrated across taxa that heat stress can cause the upregulation of detoxifying enzymes, thus exacerbating insecticide resistance or at mediating insecticide efficacy (Mack & Attardo, 2023; Perrin et al., 2022; Wang et al., 2022). Though this phenomena is not confirmed in alfalfa weevil, increased selection pressure from insecticides coupled with rising climates could be problematic for insecticide resistance management in this system. As of now, it is unclear if this shift in earlier applications is being made in response to the alfalfa plants coming out of dormancy sooner, which could be prophylactic treatment, or in response to actual weevil presence.

Resistance monitoring via bioassay is a helpful tool to understand resistance in practical terms. States such as California, who keep detailed records of pesticide applications across the state, have access to an additional tool to incorporate into resistance management protocols. Databases such as the PUR database can provide insight into pesticide usage trends over time to help answer questions about grower behavior that bioassays alone could not answer. Future studies within California could adopt this methodology of combining field data with historical pesticide usage data to further understand the patterns that contribute to resistance development. Understanding these patterns can help us create management plans that are more tailored to the specific needs of an area.

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Tables:

Table 1: Doses used in bioassays. Population column denotes doses used for suspected susceptible (S) or resistant (R) populations. Starting low dose and ending high dose differed for susceptible and resistant populations to ensure sufficient mortality to produce dose-response curves.

Dose	Dilution	$\mu\text{g}/\text{cm}^2$	floz/ac ²	Population
Control	Acetone	0	0	SR
2	1/240	0.003	0.008	S
3	1/81	0.008	0.024	SR
4	1/27	0.025	0.071	SR
5	1/9	0.074	0.213	SR
6	1/3	0.224	0.64	SR
7	1x	0.671	1.92	SR
8	3x	2.014	5.76	SR
9	27x	18.128	51.84	R

Table 2: Results from the lambda-cyhalothrin assays are shown in the table below. Results are listed alphabetically by county, the COMTRS the field was located in, and the LC₅₀ in $\mu\text{g}/\text{cm}^2$.

County	Region	COMTRS	LC ₅₀ ($\mu\text{g}/\text{cm}^2$)	Standard Error (+/-)	LC ₅₀ (floz/ac ²)	Standard Error (+/-)
Fresno	San Joaquin Valley	10M11S13E21	0.090	0.022	0.507	0.120
		10M11S13E08	0.023	0.003	0.130	0.019
Imperial	Southern	13S09S21E14	0.017	0.003	0.096	0.017
		13S09S21E14	0.016	0.003	0.090	0.21
Kern	San Joaquin Valley	15M26S24E36	0.001	0.0004	0.007	0.003
		15M28S22E24	0.002	0.004	0.009	0.020
		15M27S25E07	0.015	0.002	0.088	0.013
Kings	San Joaquin Valley	16M18S23E33	0.405	0.043	2.219	0.238
		16M18S23E32	0.631	0.074	3.462	0.407
		16M19S22E31	0.007	0.002	0.045	0.009
		16M19S21E34	0.001	0.0003	0.011	0.002

Madera	San Joaquin Valley	20M09S14E35	0.022	0.006	0.127	0.033
Merced	San Joaquin Valley	24M09S14E12	0.030	0.041	0.135	0.143
		24M10S12E13	0.010	0.003	0.059	0.014
		24M10S11E13	0.037	0.005	0.204	0.030
		24M10S12E23	0.130	0.019	0.711	0.104
Modoc	Northern	25M41N12E34	0.023	0.003	0.711	0.104
		25M42N09E25	0.008	0.001	0.050	0.008
		25M47N05E16	0.004	0.0006	0.026	0.004
		25M47N05E24	0.002	0.0004	0.033	0.007
		25M47N05E35	0.005	0.001	0.012	0.003
Riverside	Southern	33S07S23E19	1.458	0.670	7.948	3.625
		33S07S22E03	119.929	148.083	646.763	795.0625
		33S07S22E03	0.006	0.007	0.037	0.040
Sacramento	Sacramento Valley	33S07S23E03	0.022	0.005	0.127	0.028
Siskiyou	Northern	47M43N09W1 1	0.001	0.0005	0.170	0.036
		47M42N09W1 1	0.006	0.001	0.036	0.006
		47M42N09W1 4	0.001	0.0005	0.004	0.003
Yolo	Sacramento Valley	57M09N02E15	0.093	0.0156	0.508	0.084
		57M08N01W0 1	0.029	0.007	0.170	0.036
		57M09N01W3 6	0.015	0.005	0.089	0.032
		57M09N02E16	1.057	0.729	5.631	3.804
		57M08N02E03	0.058	0.010	0.322	0.053
		57M09N02E22	0.136	0.028	0.751	0.156

Table 3: Models and relevant statistics for each chemical grouping and time period of interest. Significant factors in bold, marginal significance indicated with italics.

Time Period	Insecticides	Model Adjusted R ²	Factors	F	df	p
1990-2019	Common Pyrethroids	0.36	<i>Proportion Acres Treated</i>	3.10	1	0.089
			Region	4.89	3	0.008
			<i>Prop. Acres * Region</i>	2.90	3	0.053
	Lambda-cyhalothrin	0.40	Proportion Acres Treated	9.77	1	0.004
			Region	3.73	3	0.023
			<i>Prop. Acres * Region</i>	2.89	3	0.054
2010-2019	Common Pyrethroids	0.21	Proportion Acres Treated	2.80	1	0.106
			Region	3.40	3	0.033
			<i>Prop. Acres * Region</i>	0.98	3	0.417
	Lambda-cyhalothrin	0.24	Proportion Acres Treated	4.66	1	0.040
			Region	3.08	3	0.045
			<i>Prop. Acres * Region</i>	1.13	3	0.356
2015-2019	Common Pyrethroids	0.19	Proportion Acres Treated	1.53	1	0.227
			Region	3.76	3	0.023
			<i>Prop. Acres * Region</i>	0.54	3	0.660
	Lambda-cyhalothrin	0.24	<i>Proportion Acres Treated</i>	3.34	1	0.081

Region	3.33	3	0.037
Prop. Acres * Region	0.98	3	0.422

Table 4: Quantile regression results describing changes to application timing across California regions. Models were fit separately for the 20th, 50th, and 80th quantiles. Regressions were fit as Day of Application ~ Year. Negative coefficients indicate a shift towards earlier applications, and positive coefficients represent shifts towards later application.

Region	τ	Factor	Coefficient	t	P
Northern	0.2	Intercept	-3697.70	-12.43	0.000
		Year	1.90	12.89	0.000
	0.5	Intercept	-2319.56	-14.14	0.000
		Year	1.22	15.05	0.000
	0.8	Intercept	-852.50	-5.02	0.500
		Year	0.50	5.942	0.000
Sacramento Valley	0.2	Intercept	1020.32	26.34	0.000
		Year	-0.47	-24.54	0.000
	0.5	Intercept	362.14	10.11	0.000
		Year	-0.14	-8.01	0.000
	0.8	Intercept	-554.05	-7.89	0.000
		Year	0.32	9.11	0.000
San Joaquin Valley	0.2	Intercept	466.00	28.46	0.000
		Year	-0.200	-24.54	0.000
	0.5	Intercept	-82.77	-4.11	<0.001
		Year	0.08	7.66	0.000
	0.8	Intercept	-628.29	-20.42	0.000
		Year	0.35	23.04	0.000
Southern	0.2	Intercept	1245.00	21.65	0.000
		Year	-0.60	0.029	0.000
	0.5	Intercept	1060.50	25.30	0.000
		Year	-0.50	24.01	0.000
	0.8	Intercept	72.00	1.56	0.120
		Year	<0.001	0.000	1.000

Table 5: Results of binomial logistic regression examining the relationship between the mean-centered first day of application, year, and their effect on the probability of multiple insecticide applications. Model fit was evaluated using McFadden's psuedo- R^2 . Predictor

significance was assessed using likelihood ratio statistics. Significant factors are listed in bold ($P < 0.05$)

Region	McFadden's R^2	Factor	df	LRT (χ^2)	P
Northern	0.17	First Day of Application (centered)	1	44.00	<0.001
		Year	1	8.59	0.003
		First Day * Year	1	0.50	0.481
Sacramento Valley	0.15	First Day of Application (centered)	1	10.73	0.001
		Year	11	238.22	<0.001
		First Day * Year	11	143.30	<0.001
San Joaquin Valley	0.11	First Day of Application (centered)	1	6252.2	<0.001
		Year	11	5868.0	<0.001
		First Day * Year	11	5724.7	<0.001
Southern	0.24	First Day of Application (centered)	1	8.27	0.003
		Year	23	812.96	<0.001
		First Day * Year	23	758.03	<0.001

Table 6: Year-specific slopes of the first day of application effect and the associated p-values. Original models indicated an effect of First Day of Application (centered) * Year, but the effect on secondary spray probability varied by year. Only the significant years were included. Estimated slope represents the rate of change within a given factor level.

Region	Year	Estimated Slope	SE (+/-)	p Value
Northern	2015	-0.058	0.011	<0.001
	2016	-0.068	0.009	<0.001
Sacramento Valley	2009	-0.058	0.020	0.003
	2010	-0.102	0.026	<0.001
	2013	-0.333	0.040	<0.001
	2014	-0.097	0.009	<0.001
	2015	-0.076	0.016	<0.001
	2016	-0.102	0.014	<0.001
	2018	-0.031	0.010	0.003

Region	Year	Estimated Slope	SE (+/-)	p Value
San Joaquin Valley	2019	-0.083	0.015	<0.001
	2020	-0.023	0.009	0.008
	2022	-0.041	0.017	0.018
	2023	-0.044	0.009	<0.001
	1999	-0.043	0.017	0.009
	2006	-0.017	0.007	0.019
	2009	-0.094	0.014	<0.001
	2010	-0.128	0.015	<0.001
	2011	-0.161	0.010	<0.001
	2012	-0.079	0.007	<0.001
	2013	-0.037	0.008	<0.001
	2014	-0.099	0.005	<0.001
	2015	0.012	0.004	0.002
	2016	-0.013	0.005	0.019
	2018	-0.047	0.006	<0.001
	2022	-0.063	0.009	<0.001
Southern	2023	-0.031	0.006	<0.001
	2000	-0.051	0.012	<0.001
	2003	-0.052	0.008	<0.001
	2004	-0.065	0.006	<0.001
	2005	-0.116	0.013	<0.001
	2006	-0.030	0.005	<0.001
	2007	-0.060	0.005	<0.001
	2008	-0.078	0.005	<0.001

Region	Year	Estimated Slope	SE (+/-)	p Value
	2009	-0.043	0.004	<0.001
	2010	-0.049	0.004	<0.001
	2011	-0.054	0.003	<0.001
	2012	-0.045	0.003	<0.001
	2013	-0.067	0.003	<0.001
	2014	-0.081	0.003	<0.001
	2015	-0.100	0.005	<0.001
	2016	-0.088	0.005	<0.001
	2017	-0.088	0.007	<0.001
	2018	-0.063	0.003	<0.001
	2019	-0.052	0.003	<0.001
	2020	-0.056	0.003	<0.001
	2021	-0.047	0.003	<0.001
	2022	-0.086	0.004	<0.001
	2023	-0.056	0.003	<0.001

Figures:

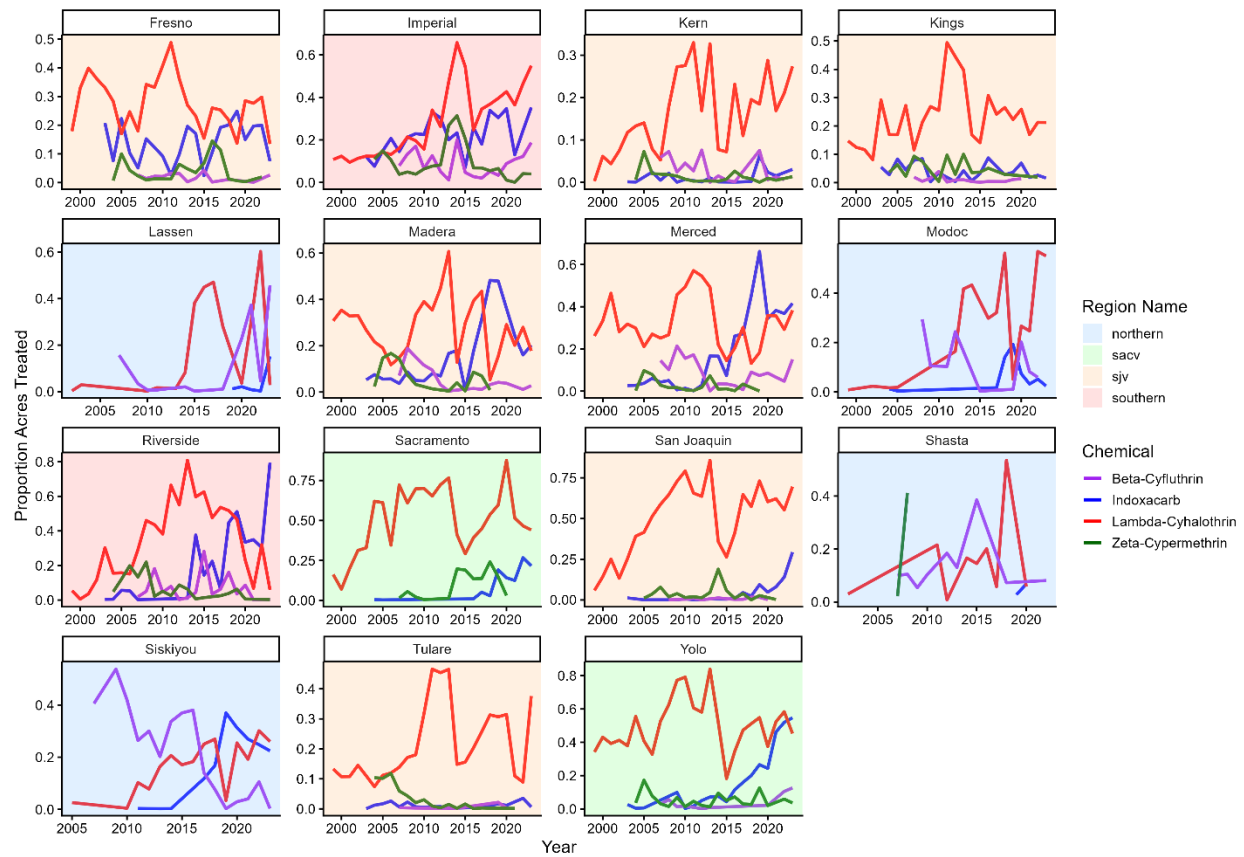


Figure 1: Graphs showing the historic use of insecticides of interest in counties where LC_{50} data was collected. Individual plots are color coded to show region. Four chemicals of interest shown here: lambda-cyhalothrin (pyrethroid; red), indoxacarb (oxadiazine; blue), beta-cyfluthrin (pyrethroid; purple), zeta-cypermethrin (pyrethroid; green).

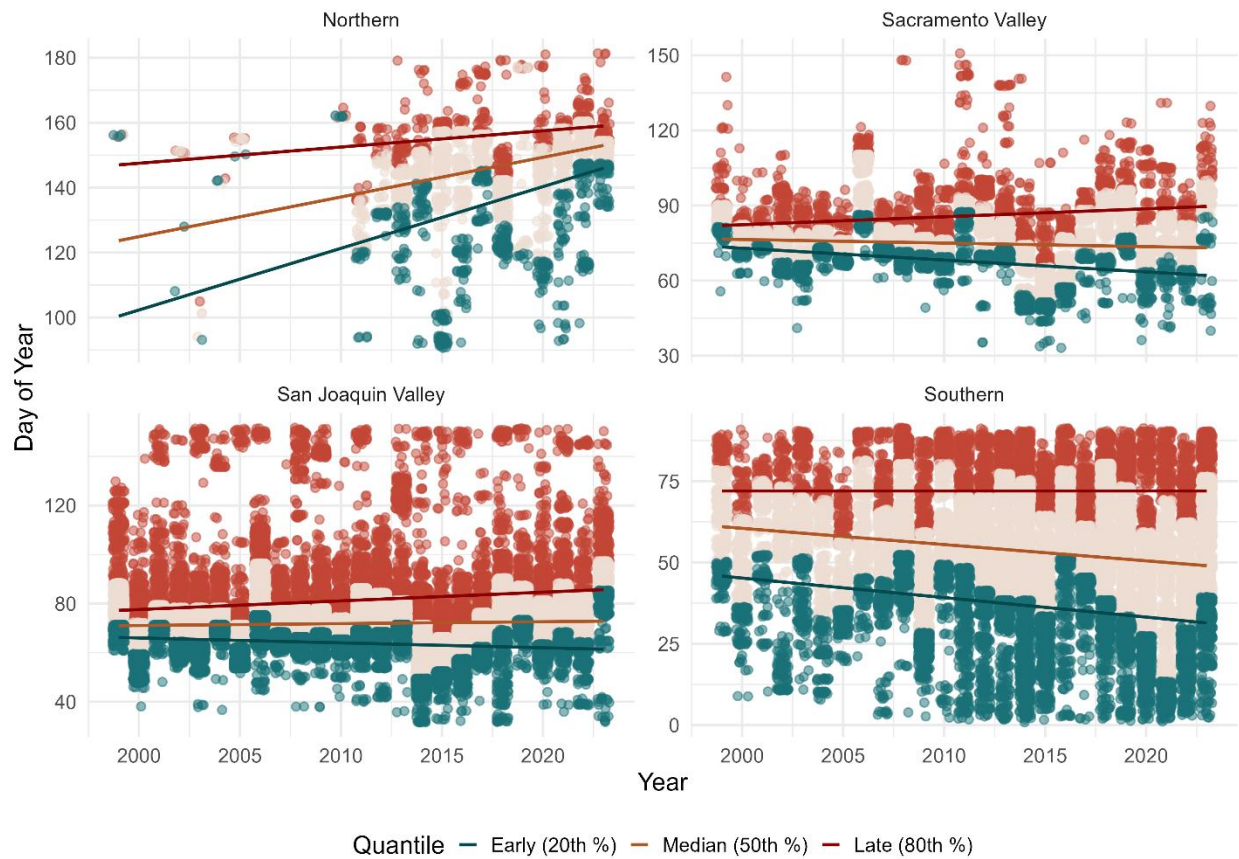


Figure 2: Quantile regression of application dates by region. Quantile regression was performed at the 20th, 50th, and 80th percentiles to generate predictive cutoff dates for each part of the season and are represented in the legend. Points in green represent the lower 20% of application dates (early), beige points represent the middle 40% of application dates (middle, or typical season), and red points represent the upper 20% of application dates (late). Early application dates in all regions except Northern have shifted forward in time, being applied earlier each year.

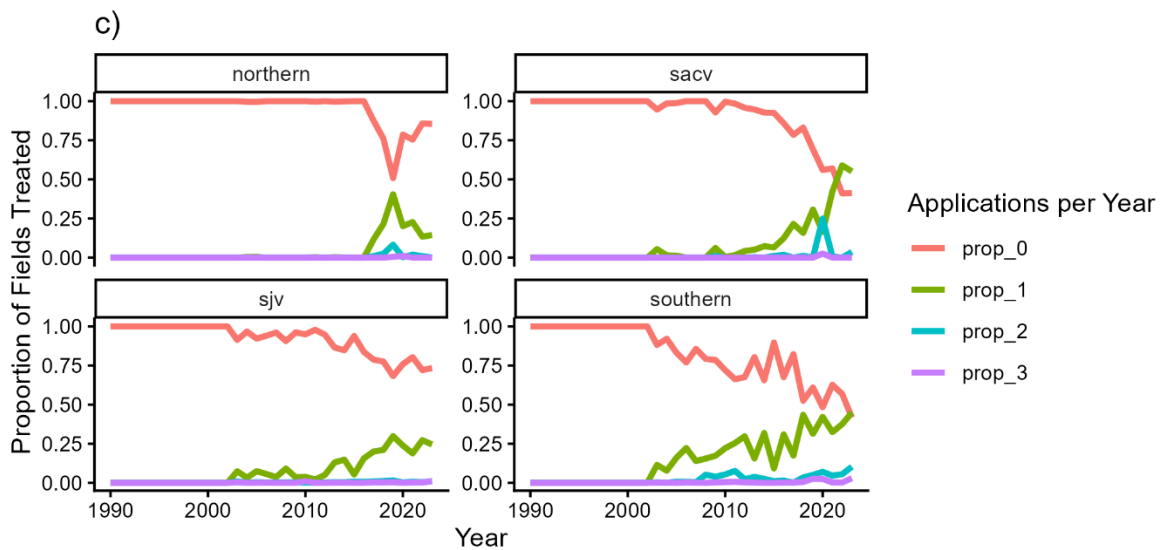
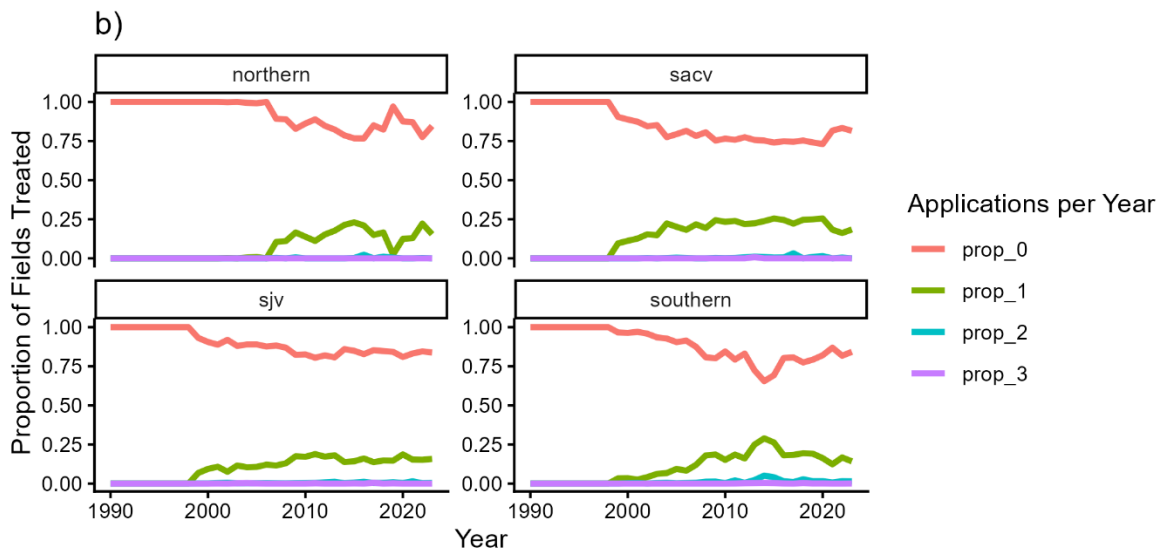
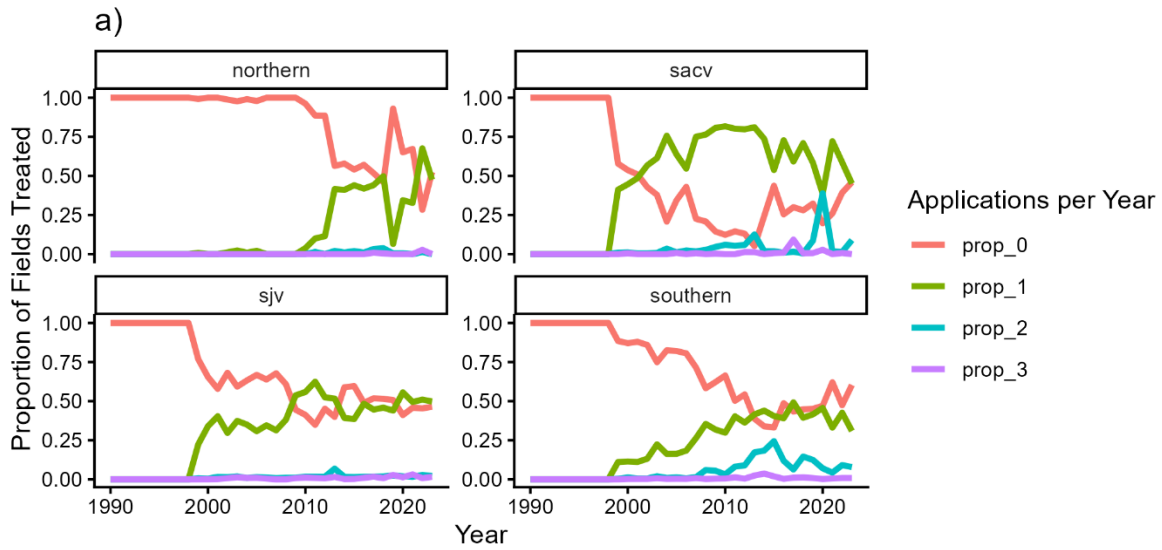


Figure 3: Proportion of fields treated with insecticides by region. a) lambda-cyhalothrin b) lambda-cyhalothrin, beta-cyfluthrin, alpha-cypermethrin, zeta-cypermethrin c) indoxacarb. Shows the proportion of fields treated 0, 1, 2, or 3 or more times (prop_0, prop_1, prop_2, prop_3 respectively) in a season each year. Proportion of 0.0 meaning no fields were treated in a particular category; proportion of 1.0 meaning all fields were treated in a particular category. Total of all categorical proportions is equal to 1.0.

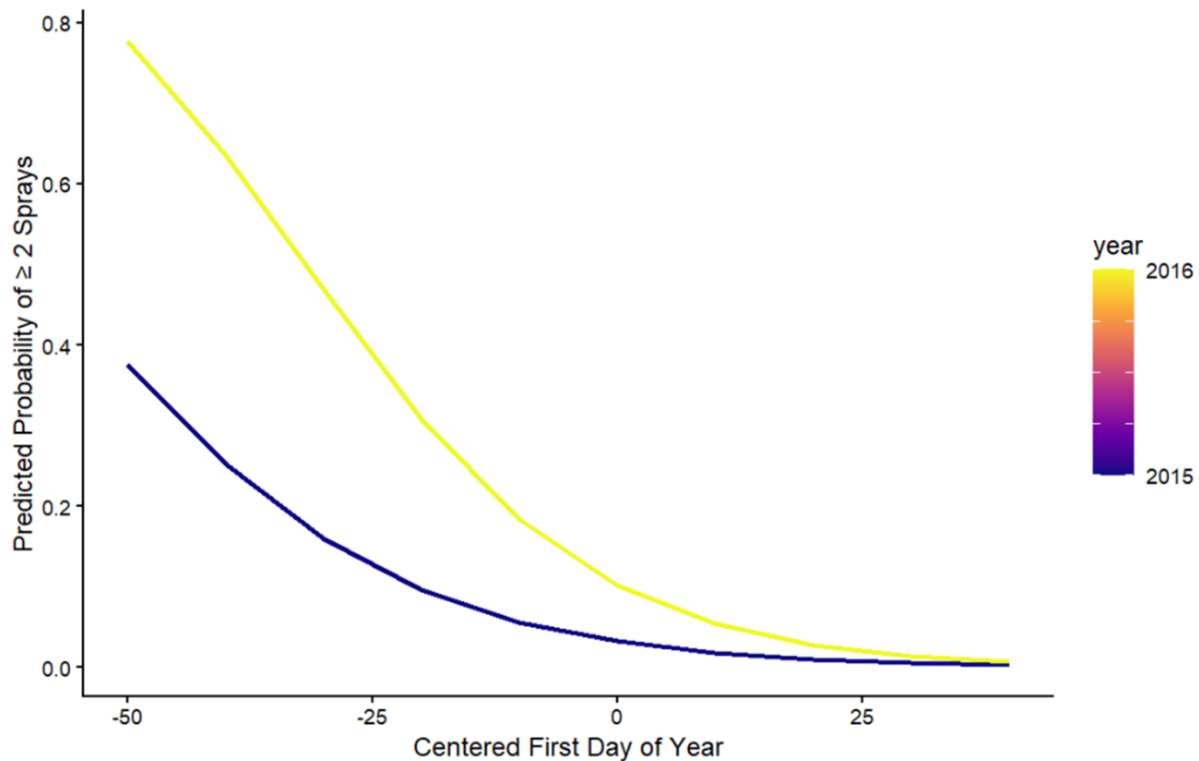


Figure 4: Predicted probability of 2+ sprays as a function of initial application timing in the Northern region. Plot shows the year specific predicted trends of significant years. Non-significant years were excluded from this plot ($p \geq 0.05$). Line color indicates year. Negative values of Centered First Day represent applications that occurred earlier than the mean application date.

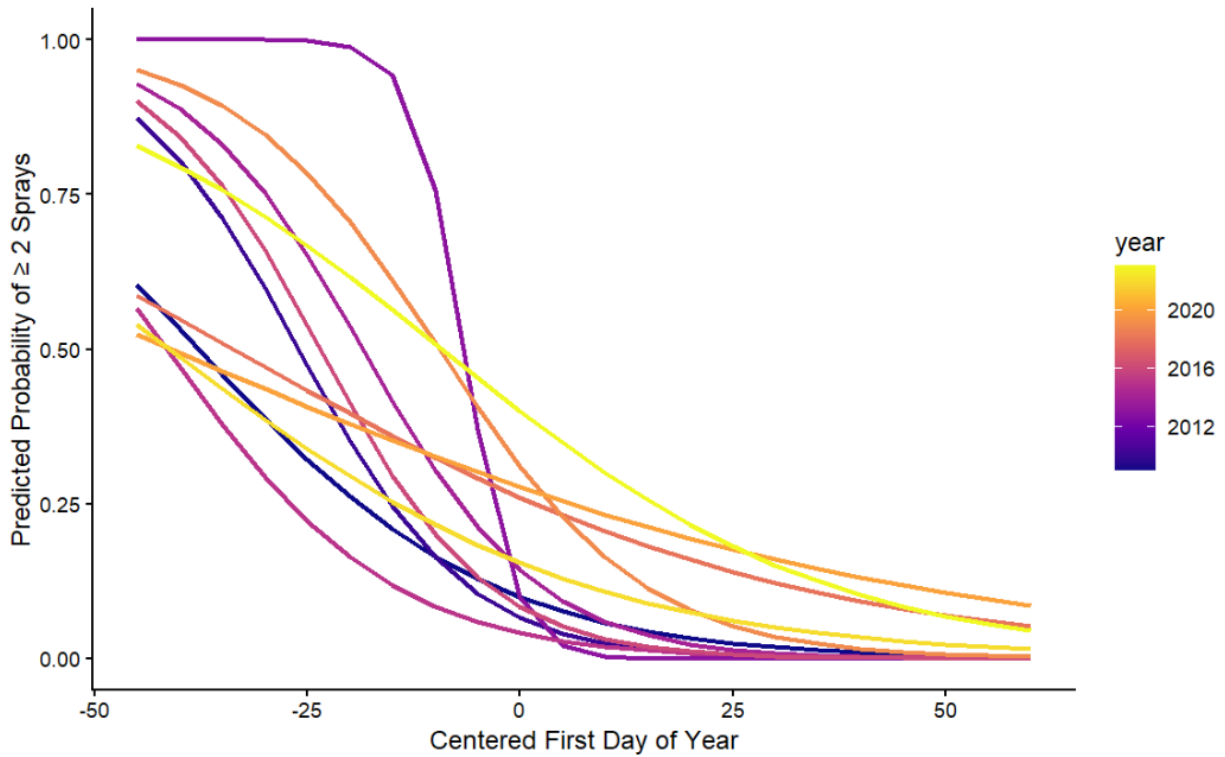


Figure 5: Predicted probability of 2+ sprays as a function of initial application timing in the Sacramento Valley region. Plot shows the year specific predicted trends of significant years. Non-significant years were excluded from this plot ($p \geq 0.05$). Line color indicates year. Negative values of Centered First Day represent applications that occurred earlier than the mean application date. All years in which this relationship was significant resulted in a predicted probability of secondary sprays of at least 50%, though this relationship varied among years.

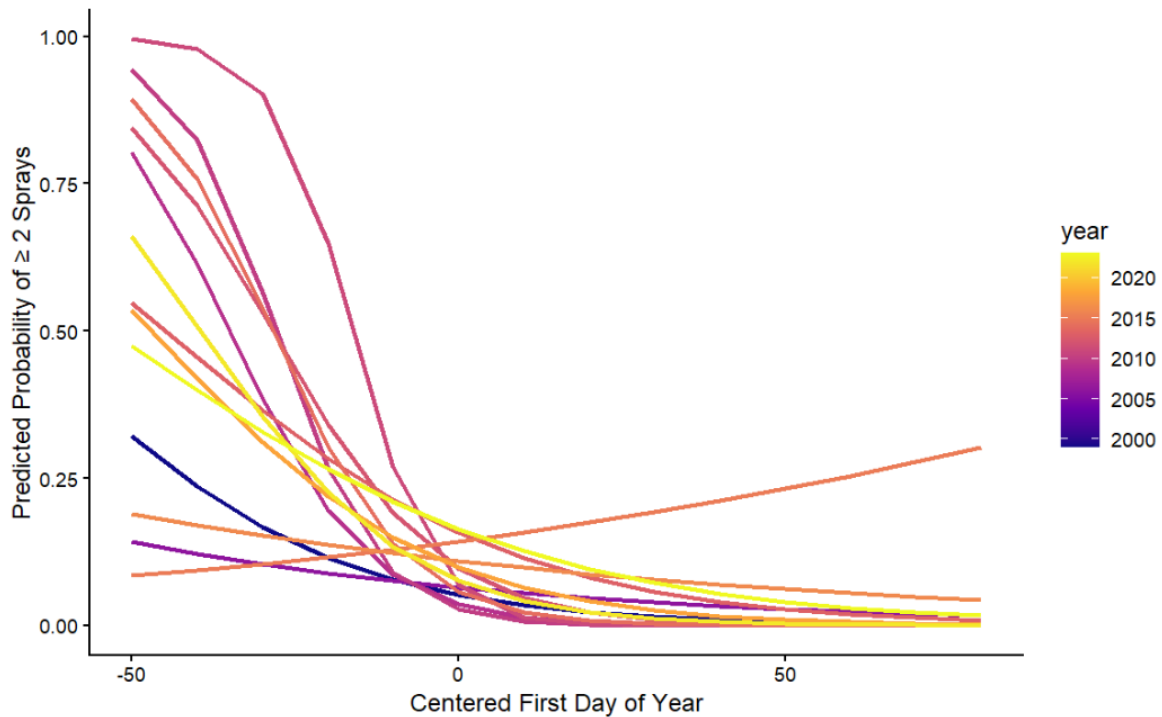


Figure 6: Predicted probability of 2+ sprays as a function of initial application timing in the San Joaquin Valley region. Plot shows the year specific predicted trends of significant years. Non-significant years were excluded from this plot ($p \geq 0.05$). Line color indicates year. Negative values of Centered First Day represent applications that occurred earlier than the mean application date. There was variation amongst significant years in how strong of an effect this relationship had on the probability of secondary sprays. The relationship in 2015 was significant, however the relationship was reversed in this year, meaning that there was a higher correlation between late sprays and secondary sprays.

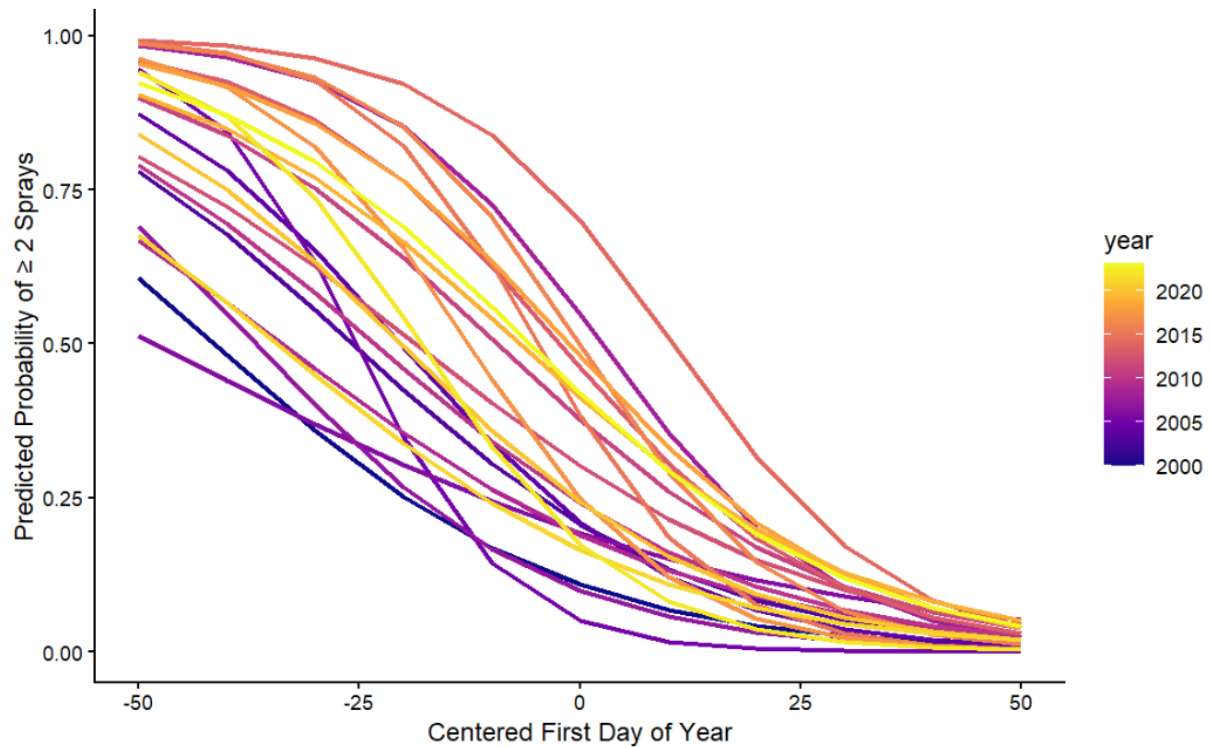


Figure 7: Predicted probability of 2+ sprays as a function of initial application timing in the Southern region. Plot shows the year specific predicted trends of significant years. Non-significant years were excluded from this plot ($p \geq 0.05$). Line color indicates year. Negative values of Centered First Day represent applications that occurred earlier than the mean application date. All years in which this relationship was significant resulted in a predicted probability of secondary sprays of at least 50%, though this relationship varied among years.

Evaluating the damage potential of tadpole shrimp (*Triops longicaudatus* (LeConte)) on California rice (*Oryza sativa*) under different conditions

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Key words:

ABSTRACT

California rice fields are but one example of a crop system in which integrated pest management options are limited. In the first few weeks of the growing season, growers must contend with tadpole shrimp (*Triops longicaudatus* (LeConte)), the primary pest of rice. Tadpole shrimp have desiccation resistant eggs that hatch when introduced to water. By the time rice is planted in the fields, tadpole shrimp are already present and capable of damaging the crop. The individual growth and development of these organisms is reasonably well understood, but their development in tandem and the resulting damage potential under varying conditions has not been made clear. To address this foundational gap and improve IPM in this system, we performed growth chamber and greenhouse mesocosm experiments to determine the effects of planting timing, TPS density, and temperature on this system. We found that higher temperatures increase the rate of rice development but also increases the amount of damage that TPS inflict. As rice develops faster, it shortens the window of time that growers must contend with the shrimp. However, this is a double-edged sword, particularly in cases of severe infestation, as it also reduces the amount of time a grower has to act to protect their crop. This experiment helped to elucidate TPS damage under different conditions which, as the climate becomes more extreme and unpredictable, will help growers make better management decisions.

Introduction:

California is a major rice production zone in the United States with unique climate and production practices relative to other areas, which lends itself to unique pest problems. The majority of California rice is water-seeded (Hill et al., 2006; Rutger & Brandon, 1981). In a water-seeded system, rice fields are flooded and airplanes are used to fly pregerminated rice seeds onto the field. The pre-germination process involves soaking the rice seed in water for 24 hours to begin the germination process and help the seeds sink to the bottom of the basin once planted (Rutger & Brandon, 1981; Tindall & Fothergill, 2012). The flooding process can take multiple days depending on the size of the field, with a 100-acre field possibly taking four to five days to flood (Rutger & Brandon, 1981). During this flood-up period, a key pest of California rice, the tadpole shrimp (Notostraca, *Triops longicaudatus* (LeConte)), may be hatching.

The tadpole shrimp, hereto referred as TPS, are freshwater crustaceans that have evolved to live in vernal, or temporary, water sources. They are native to California and thrive in the temporary wetlands created by rice production. One key to their survival between floods is their desiccation-resistant eggs. TPS eggs can lay dormant for extended periods of time until the soil is flooded again and they are re-hydrated (Grigarick et al., 1985; Rogacki & Brysiewicz, 2021). TPS will begin hatching within 24 hours of submergence, and 24 to 48 hours later, the filter feeding neonates emerge (Rogacki & Brysiewicz, 2021). After their first molt, they resemble the adult form and are fully omnivorous, eating both plants and other organisms, at times even cannibalizing other TPS (Grigarick et al., 1985; Bloese et al., 2024; Rogacki & Brysiewicz, 2021; Tindall & Fothergill, 2012).

TPS will feed directly on the roots and leaves of aquatic plants, including the freshly planted rice seedlings, damaging rice stands (J. B. Bloese et al., 2021) . Their foraging behaviors

cause multiple problems for young rice stands. First, as they root around in the mud, they risk dislodging the seedlings (Grigarick et al. 1985). Water-seeded rice has a very weak root system in the first few weeks after planting (Tindall & Fothergill, 2012). Dislodged plants will often float to the surface and collect at the edges of the field (Grigarick et al. 1960). Severe infestations of tadpole shrimp can greatly reduce stand count, thus impacting yield (Tindall & Fothergill, 2012). Its destructive feeding habits are what makes the TPS a key early season pest of rice, but the desiccation resistant eggs are what provide the TPS a biological advantage against management tactics. Because fields are flooded prior to planting, and there may be additional delays that increase the time between flood and planting, the TPS may have a developmental headstart on the rice that growers must contend with.

TPS only pose a threat to rice for a short window of time, which means management decisions should be made quickly in case of infestations. However, scouting for TPS can be difficult, since they spend most of their time feeding in the muddy bottom of rice fields as opposed to swimming freely in the water column (J. B. Bloese et al., 2021). Growers may look for floating seedlings or murky water, especially in fields with known infestation histories (Espino et al., 2024). Growers can also inspect a 1 ft² area and count healthy seedlings to ensure an average of 25 seedlings per ft² for optimal yield at harvest. Otherwise, growers are recommended to treat if there is also observed evidence of TPS (Espino et al., 2024). Once the roots of the rice are established, rice is generally safe from TPS damage, limiting its pest status to the first two to three weeks of the season (Espino et al., 2024). This short period of activity means growers, if they decide to treat, should react quickly to the first signs of shrimp activity to mitigate damage. Many growers tend to treat this pest prophylactically (J. B. Bloese et al., 2021). Prophylactic treatments have the benefit of managing an infestation before it gets severe but can

have long term consequences. Applications made in fields with little to no TPS presence can be inefficient and unnecessary expenditures. The risk of resistance development is also increased with repeated usage (J. B. Bloese et al., 2021). Effectively controlling a pest in a short period of time is a challenge and numerous environmental and production factors may alter the intensity of infestation and resulting damages.

Growers can manage flooding and planting timing, but cannot control environmental factors such as temperature or the infestation rate of a particular field, all of which impact pest management. The growth and development of rice under different temperature conditions is reasonably well modeled and documented. The optimal temperature range for TPS growth is, likewise, fairly established in the literature. The relationship between the two under varying conditions is less clear. Specifically, the damage potential is not yet defined. Climate change is also an issue that California growers will have to contend with. Climate instability already threatens the amount of water available to California rice growers (Jackson et al., 2011; Pathak et al., 2018; Reiter et al., 2018). Water temperature is also directly related to air temperature. Radiative heating from the sun can often cause the water to exceed air temperature, especially in the early season before the rice canopy develops and the basin is highly exposed (Kuwagata et al., 2008). As ectotherms, arthropod development is sensitive to temperature and, in many species, warmer temperatures can increase the rate of development at the cost of smaller size at maturity (Atkinson, 1995; Daufresne et al., 2009). Similar to invertebrates, the early stages of plant growth are also influenced by temperature and tend to develop faster in warmer conditions (Atkinson 1995). Climate change has already altered the development and phenology of many pest species (Forrest, 2016; Skendžić et al., 2021). When considering these developmental changes in the context of short management windows, increased TPS growth rates could give

growers even less time to decide on and implement treatment plans. It is also unknown if what effects, if any, warmer conditions will have on the overall potential damage incurred to the rice. A deep understanding of the fundamental biology of a pest, its host, and their interaction is critical for the development of more effective IPM programs (Stern et al., 1959) and clarifying these relationships is the first step in helping growers better manage these pests.

In this study, we aimed to examine the damage potential of TPS in rice under varying temperature, planting, and pest density conditions. To accomplish this, we performed two experiments addressing two objectives: Objective 1) determine the damage potential under different temperatures, TPS densities, and planting timings in a growth chamber setting; and Objective 2) determine the damage potential under different temperatures and TPS densities in a greenhouse mesocosm setting.

Materials and Methods:

Growth chamber:

This experiment used PHCBI MLR-352H-PA environmental test chambers (PHCbi) to maintain constant temperature and replicate field light conditions. Small bins approximately 17.78 cm by 45.72 cm were used as the experimental unit. This was a full factorial design experiment in which we manipulated temperature, planting timing, and shrimp density. We tested a range of temperatures, representing a “cool,” average, and “hot” planting season. The temperatures tested were 23.8 °C, 26.7 °C, and 29.4 °C respectively. The cool temperature was based off the lowest regional, seasonal average temperature in the past 10 years. The “hot” temperature was similarly decided based on the highest temperature in the past 10 years, plus an additional 2 °C increase as predicted by climate change models. We tested 2 planting timings,

early and late. Finally, we tested a low and high density, calculated based on field rates of 60 tadpole shrimp per square meter. Treatment specifications can be found in [Table 7](#). Five replicates were attempted of each temperature, two replicates from each treatment were dropped either due to high algae levels or premature shrimp death.

Approximately 2.5 cm of soil was placed into the bins, then flooded. Tadpole shrimp eggs were added directly to the bins at this point, to simulate field hatching conditions, and excess shrimp were removed after hatching. Rice was soaked in water to pre-germinate for 24 hours prior to planting. At planting, 30 seeds were placed into each bin. In the high shrimp density treatments, mesh barriers were placed to separate the shrimp to prevent cannibalism. Each section was planted with 10 seeds to approximate optimal rice stand conditions. Data were recorded every other day after planting until harvest, about two weeks from planting or until the majority of plants in the controls breached the surface. Shrimp were carefully removed and photographed for digital measurement using ImageJ. We randomly sampled ten plants for growth measurements, and plant stage is recorded. At harvest, remaining shrimp were measured and plants were measured for root and shoot length and biomass.

Growth chamber data analysis:

We analyzed data using R version R version 2022.12.0+353. Rice height was averaged across a subset of plants within each replicate each day and analyzed using a linear mixed model using the *lmer* function in the lme4 package (Bates et al., 2015). Mean length was $\log_{10}$ transformed and analyzed as a response to the interaction of two fixed effects and one random effect. Our interaction term was treatment, a combination of both temperature and density, and sampling point. The bin was included as a random effect to account for repeated measurements. Shrimp size over time was analyzed using the same approach. Pairwise comparisons were conducted

using estimated marginal means with Sidak's adjustment ($\alpha = 0.05$). For biomass and stand count, the two sampling periods were analyzed independently and treatment effects were evaluated using Kruskal-Wallis tests. We ran separate tests to test the effect of density and temperature. Pairwise Wilcox tests with a Benjamini-Hochberg adjustment ($\alpha = 0.05$) were performed to find differences between treatments. To analyze root length, shoot length, and shrimp size at harvest, we used the same method across metrics. We averaged harvest measurements across treatment and analyzed the data using a linear model. Shoot length was $\log_{10}$ transformed. Fixed effects included the interaction of timing, temperature, and density, and replicate. We used estimated marginal means, using the *emmeans* package (Lenth 2023), with a Sidak correction to make pairwise comparisons.

Greenhouse:

We conducted a greenhouse experiment in the greenhouses at UC Davis. We used 10-gal plastic storage totes as the experimental units. This was a factorial design experiment testing two temperature treatments and three shrimp density treatments. Our low temperature was 23.8 °C and our high was 29.4 °C. We tested TPS densities of three and five TPS, as well as a zero TPS control. Refer to (Table 1) for full treatment details. We conducted five replicates of this experiment. Greenhouse temperatures were set to 24 °C to maintain ambient temperature conditions to reduce variability within experimental units. Individual bins were heated using 50 watt aquarium heaters (Dattoo). Low temperature treatments were heated with one aquarium heater, while high temperature treatments required two. Heaters were plugged into temperature control regulators (Pymeter; PY-21TS), which monitor water temperatures and turn the heaters on or off to maintain the target temperature. Controllers were set to turn on 1.8 °C below the target temperature, and off 1.8 °C above.

Bins were filled with approximately five cm of dirt, and heaters were placed roughly at the center of the bins. Bins were flooded to a depth of about 8 cm, and separate bins were simultaneously flooded with dirt containing shrimp eggs. Tadpole shrimp were allowed to hatch and develop separately outside of the experimental bins at ambient greenhouse temperatures to ensure that each bin had a precise number of shrimp at the start. We transferred them when they were large enough to survive transfer, but too small to damage rice. Shrimp were moved based on a size of 0.3 to 0.5 mm, which typically occurred 5 days after flood. Rice was pre-germinated the day before flood and planted the day after, for a total of 48 hours of pre-germination. This still aligns with grower practice, is more realistic to the field, and aimed to achieve stand establishment under higher shrimp pressure. Each bin was planted with 100 seeds at the same time, 1 day after flood.

Plant data were recorded in the control units with no shrimp because shrimp stir and muddy the water and make direct plant measurement difficult to impossible. Plant data were taken two days after planting and then every two to three days until 75% of the plants were at least 3cm taller than the designated water depth. If the control plants hit that threshold, all bins in that temperature treatment were harvested. For example, if the 23.8 °C control reached that threshold, the 23.8 °C low and high density treatments were harvested as well. The number of plants in each growth stage was recorded, and 10 plants were randomly sampled for height. Because shrimp presence can muddy water, data were only recorded for controls during the experiment. Control data were used as a proxy for growth in other treatments. At harvest, growth stage, biomass, stand count, and root and shoot length of all treatments was recorded. Biomass was recorded combined for all plant material. Stand count was recorded as the total number of plants. Shrimp were randomly sampled for size at the beginning of the experiment. They were

placed, then sampled again three to four days later, typically maintaining a Monday/Friday (or equivalent) sampling window. This was to ensure shrimp wellbeing through the duration of the experiment. Shrimp were carefully removed from the bins and subsampled, placed in a tray with water, then photographed to be measured digitally with ImageJ. At harvest, bins were drained and all remaining shrimp were recorded.

Data analysis

To analyze rice height over time, rice height was averaged across plants each day and analyzed using a linear mixed model in R. The model was fit using the *lmer* function in the lme4 package (Bates et al., 2015). Mean height was $\log_{10}$ transformed. Fixed effects included an interaction between our treatments (temperature and density) treated as a single factor and sampling point as well as replicate. The bin was included as a random effect to account for repeated measurements. Shrimp size over time was analyzed using the same approach. Significance of main effects was tested with a Type III analysis with Satterthwaite's adjustment using the *Anova* function in the car package (Fox and Weisberg, 2019). Pairwise comparisons were conducted using estimated marginal means with Sidak's adjustment for multiple comparisons ($\alpha = 0.05$). Biomass and stand were analyzed using linear models. Fixed effects included the interaction of temperature and density, their main effects, and block. Post hoc analysis was conducted with estimated marginal means with Sidak's adjustment. To analyze root length, shoot length, and shrimp size at harvest, we averaged measurements taken at harvest and analyzed the data using linear models. Fixed effects included the interaction of temperature and density, temperature, density, and replicate. We used Tukey's HSD test with a Sidak correction to determine treatment differences and used estimated marginal means to make pairwise comparisons. Root, shoot, and shrimp size were analyzed separately using the same methods.

Results:

Growth Chamber:

Rice growth stage and height: We found that the interaction between the treatments (treated as a combination of factors) and sampling point was significant for height ($F_{5,77} = 20.21$, $p < 0.001$). The plants in the 23.8 °C Timing 2 treatment were significantly shorter than plants in the 29.4 °C Timing 1 treatment. The remaining, intermediate treatments did not significantly differ from each other. However, the 23.8 °C Timing 1 treatment was most similar to the other timing treatment in the same temperature. Plants in both 26.6 °C timing treatments and the 29.4 °C Timing 2 treatment were, on average, taller than the 23.8 °C treatments but shorter than 29.4 °C Timing 1.

Rice biomass and stand count: For both metrics and both timings, the control plots had a significantly higher biomass and stand count than shrimp density treatments. With biomass, we found that density was a significant factor ($\chi^2 = 21.83$, $df = 2$, $p < 0.001$). Within Timing 1, both density treatments were significantly different than the control, but not from each other. Within Timing 2, density was again significant ($\chi^2 = 23.98$, $df = 2$, $p < 0.001$), differing from the controls but not each other. Similarly, for stand count, we found that density was significant in both timings ($\chi^2 = 21.95$, $df = 2$, $p < 0.001$; $\chi^2 = 20.09$, $df = 2$, $p < 0.001$), and that both density treatments differed from the control but not each other.

Rice root and shoot length: For both metrics, shrimp density was significant (Root: $F_{2,18} = 19.62$, $p < 0.001$; Shoot: $F_{2,18} = 8.45$, $p = 0.003$). The controls and the 3 density treatments did not differ from each other, but lack of harvest data in several 1 TPS density treatments due to severe shrimp herbivory made the results inconclusive.

Shrimp growth: Timing had a marginal effect on shrimp size ($F_{1,168} = 3.80$, $p = 0.061$), but post-hoc comparisons did not identify significant differences between treatments. Density had a significant effect ($F_{1,168} = 11.60$, $p = 0.002$), and the two density treatments were significantly different from each other with the single shrimp density larger on average.

Shrimp at harvest: Temperature and density had a significant effect on shrimp size ($F_{2,27} = 8.04$, $p = 0.002$; $F_{1,27} = 13.06$, $p = 0.001$). Shrimp in the high temperature were on average larger, but shrimp in the low and medium temperatures did not differ significantly in final size.

Greenhouse:

Rice growth stage and height: For growth stage, while we did not analyze the data formally, at every day, the high temperature controls were further along in the developmental process than the cold temperatures, indicating faster development in warmer water (**Figure 20**). At harvest, a higher proportion of the high temperature plants reached the V3 growth stage than in the low temperature (**Figure 21**). In the high temperature treatments, the control and low TPS density treatments tended to have more in the V3 stage relative to the high TPS density. Conversely, the lower temperature treatment had higher proportions of V3 plants in the control and high TPS density treatments. In regard to plant height, day number had a significant effect on plant height ($F_{3,31} = 249.14$, $p < 0.001$; **Figure 22**), effectively meaning that in both treatments, plants grew taller each day. The interaction between temperature and day number had only marginally a significant effect on plant height ($F_{3,31} = 28.21$, $p = 0.006$; **Figure 22**). Plant height in each temperature treatment differed each day, and the two temperatures were significantly different from each other, with the 29.4 °C temperature having larger plants on average.

Rice biomass and stand count: We found that temperature ($F_{1,23} = 12.76, p = 0.00;$) and shrimp density ($F_{2,23} = 8.98, p = 0.001$) both had an effect on total biomass, but the interaction between the two was not significant ($F_{2,23} = 1.88, p = 0.176$, **Figure 16**). Our two temperatures were significantly different from each other, with the 29.4 °C treatments having higher biomass on average. Our control treatment biomass was different from our shrimp density biomasses, and on average heavier. There was also a greater percent difference in biomass in the high temperature treatments than in the low temperature treatments. In the high temperature treatment, the biomass in the high TPS density treatment was 68.25 percent lower than in the controls, and the low TPS density treatment was 60.61 percent lower. Compared to the 64.40 and 62.42 percent difference respectively in the cold temperature treatment, the high temperature seemed to incur a greater amount of damage at high TPS densities. High and low shrimp density biomass did not vary from each other. Shrimp density had a significant effect on average stand count ($F_{2,22} = 13.37, p < 0.001$), and stand counts at both densities were significantly different from the controls, but did not differ from each other (Figure 10).

Rice root and shoot length: We saw a significant effect of density on average root length ($F_{2,22} = 14.13, p < 0.001$; **Figure 18**), with the control treatments having longer roots on average than either TPS density treatment. The high and low shrimp density treatments in both temperatures did not differ significantly from each other. In average shoot length, we saw that temperature had a significant effect ($F_{1,22} = 27.92, p < 0.001$), and that the high temperature plants had longer shoot lengths than the low temperature plants (**Figure 19**).

Shrimp growth: TPS starting size ranged from 0.413 to 0.495 prior to Day 1 in **Figure 23**. Starting size of each replicate can be found in **Table 9**. Only day had a significant effect on size

($F = 26.13$, $p < 0.001$), and average shrimp size varied at all 3 sampling points, though not significantly.

Shrimp at harvest: Only density had a significant effect on shrimp size ($F_{1,12} = 12.43$, $p = 0.004$; **Figure 23**). High and low densities differed at both temperatures, with the low density shrimp being larger on average.

Discussion:

This study aimed to determine the damage potential of TPS under different environmental and planting conditions. Our greenhouse study demonstrated that rice grew faster in warmer conditions but still incurred more damage from TPS as in cooler conditions. Final TPS size appeared to be heavily influenced by population density. We did see size differences as an effect of temperature in our growth chamber experiment (**Figure 8**), but this effect was not observed in the greenhouse study (**Figure 23**). Ultimately, while we did show the effect of various factors on damage potential, there are some limitations to these studies that will be discussed.

Our growth chamber experiment showed little difference in yield or damage because of temperature or planting timing treatments. Differences in yield, biomass, root damage, and shoot damage are largely attributable to the presence or absence of TPS. The TPS did differ in size as a result of both density and temperature effects. The low density shrimp were larger across all treatments and planting timings. However, this study had some limitations that must be considered when interpreting the results. First, the high density TPS treatment used custom dividers to split the area available to the shrimp into even sections $\frac{1}{3}$ the size of the area, with

each shrimp having access to 10 of the 30 total seedlings. Dividers were used to prevent cannibalism among TPS and to reduce seedling availability to individual shrimp to simulate high pest density. Because TPS could not move between compartments, mortality within a compartment left the rice seedlings inaccessible to remaining TPS. Early mortality reduced overall herbivory pressure and may have artificially increased seedling survival at high TPS density. The increased survival rate would imply that higher TPS densities are less damaging to rice, but that would not be the case in a field setting.

In regards to our greenhouse study, rice growth and development varied significantly by temperature, but germination and stand count were not negatively affected. Our control plots, which lacked TPS, allowed us to see how the plants developed independently in the given conditions. Rice plants in the high temperature treatment progressed through growth stages more rapidly, which resulted in a higher biomass at the experiment termination. Multiple field and modeling studies corroborate our mesocosm study for these metrics and show that rice development rates increase with water temperature, until 30 to 35 °C depending on variety (Sharifi et al. 2018; Herath and Ormrod 1965; Owen 1971). Alternatively, stand count was unaffected by temperature. The similarities in stand count in both temperature treatments show that temperature did not affect germination. Our temperatures were well situated between the minimum and maximum temperature for rice germination, 18 °C and 45 °C, outside of which rice will not germinate (Owen 1971). Within the acceptable germination temperature range, rice will germinate more slowly in lower temperatures (Owen 1971; Sharifi et al. 2018). In totality, the rice did perform better in warmer conditions, as evidenced by the separation in size by the second sampling point, and speed at which the warm treatment advanced through growth stages and accumulated biomass. Historically, California rice production has been so successful

because May temperatures in California are optimal for seeding (Caton et al. 1997). The temperature range we assessed encompassed a 10-year historic low planting temperature and an extreme climate prediction. Our results show that, as climate change and variable spring temperatures intensify, stand establishment will be less of a concern for growers.

Conversely, whereas rice growth seemed to be highly dependent on temperature, shrimp growth in the greenhouse experiment seemed more dependent on population density. All TPS used in this experiment began at approximately 0.5cm in size and, though there was variability in size at experiment termination, there was no significant difference in size between treatments during the duration of the shrimp in the low density treatment were larger on average than the high density shrimp. These size differences are likely attributable to resource competition. The TPS densities used in this trial were calculated based on a field density of 60 TPS per 1 m², which would be considered severe infestation. In a field setting, severe infestations may result in smaller shrimp due to competition, however the damage incurred seems to be independent of pest size. Higher population densities as the cause for increased competition is well documented in insects and other arthropods to result in delayed maturity, reduced adult size, and life history costs (Agnew et al. 2002; Mueller 1997), and severe infestations might have deleterious effects for the overall TPS population over time. The difference in size as an effect of temperature was not significant in this trial, but there was a significant difference in shrimp size in our growth chamber experiment. Namely, the lowest and highest temperature treatments, which matched our greenhouse temperatures, were significantly different from each other at each planting timing. Multiple studies documenting TPS development have noted the importance of temperature in their developmental cycle, particularly their dependence on high temperature (Scott and Grigarick 1979; Scholnik 1995). In the growth chamber, we were able to hold the temperatures

at a constant as opposed to the greenhouse which experienced a daytime temperature peak. While the temperatures average out to the target temperature in the greenhouse, this fluctuation may have dampened the effect of temperature on shrimp development since the TPS were exposed to nearly the same temperature for the same amount of time every day even though night time temperatures differed. In both experimental settings, there were pronounced differences in shrimp size when examining the effect of density. In a field setting, it is possible that both temperature and density have an effect on the overall development of TPS. The temperature-size reduction hypothesis states that higher temperatures can cause arthropods to develop more quickly at the cost of reduced size-at-stage or, in other words, stunted growth (Atkinson 1995; Aquilar-Alberola and Mesquita-Joanes 2014). What matters more for management than size at maturity is how quickly the TPS hatch and develop into a size stage that can cause appreciable damage to the rice.

We found that the rice responded to both temperature and TPS presence, but those factors did not interact with each other. TPS density had an effect on stand count, but only the control treatment differed significantly from the other density treatments ((Figure 17). Essentially, stand count was only affected according to if the treatment contained shrimp or not. Similarly for biomass, control plots that had no shrimp had significantly higher biomass than both shrimp density treatments only because there was no herbivory damage (Figure 16). Root size is heavily impacted by TPS presence, but we did not see differences amongst the tested densities. The differences we saw only reflect the presence or absence of shrimp. Root length was fairly comparable between both temperature control treatments. Water seeded rice has more vulnerable roots than drill seeded or transplanted production methods (Tindall & Fothergill, 2012). Root damage and stand count was consistent and highly comparable between temperature treatments.

On the other hand, shoot length was heavily impacted by temperature. The high temperature plants, particularly in the control treatment, had a higher median length than the cold temperature plants. The high temperature treatments at both shrimp densities had higher median lengths and more variability than the cold temperature plants. Perhaps more important than the length of plant shoot is how much quicker the warm temperature rice grew than the cold treatment.

As the temperatures increase and water availability becomes less predictable in response to climate change, the way we respond and manage our rice systems must also be ready to adapt and change. There are varying opinions on how exactly rice production could be affected as the Central Valley becomes more impacted by climate change. Several studies predict yield loss, with predicted loss of a minimum of 10% up to 41% in the 2070s (Lee et al., 2011; Li et al., 2024). Historically, California's May weather has been ideal for the early development of rice (Caton et al., 1998), but May heatwaves may reduce yield up to 10% (Jackson et al., 2011). Though we demonstrated faster development and higher biomass accumulation at higher temperatures, our experiment did not follow through until harvest. Additionally, we were only able to effectively manipulate water temperature, which is more important for the early development of the plant (Shimono et al., 2007). Without taking the plants all the way to harvest while adjusting air temperature, it is not clear if this accelerated development will have a positive or negative impact on yield. In previous drought years, flooding of rice fields was often delayed due to water availability (Reiter et al., 2018). Drought or unpredictable precipitation, reduced snowpack in the Sierra Nevada, and changes to water allocation to adapt to a new climate could result in more delayed floods, putting growers at higher risk for elevated temperatures at or around planting (Jackson et al., 2011; Joyce et al., 2011; Medellín-Azuara et al., 2008; Pathak et

al., 2018; Reiter et al., 2018). Delayed flood and planting puts growers at higher risk for TPS damage in the early season, and yield loss later on.

Though there are certainly limitations to our studies, as discussed, the biomass reduction suggests that damage directly from TPS may worsen somewhat with increasing temperatures. It is understood that one potential effect of climate change is elevated CO₂ levels (Eigenbrode & Adhikari, 2023; Hussain et al., 2020). Elevated levels of CO₂ can cause plants to fix more carbon in relation to nitrogen, thus leading to lower relative levels of N levels. This reduced nutrient content can sometimes result in higher herbivory rates by arthropod pests to make up this deficit (Hamman et al., 2021). The differences in predicted rice yield loss can be attributed to how a particular model factored in CO₂ emissions. Elevated CO₂ can increase biomass and partially offset yield loss, but reduced nitrogen assimilation under these conditions may increase susceptibility to TPS herbivory (Li et al., 2024; Jackson et al., 2011; Lee et al., 2011). Enhanced plant growth could potentially compensate for damage at low infestation levels (Zvereva & Kozlov, 2006). The window for management, however, may shorten as rice develops faster. Growers would have to be more diligent with scouting programs to make proper management decisions. With less time to act, growers may find it more justifiable to treat prophylactically to reduce risk, as they already tend to do (J. Bloese et al., 2024). As a response, this would functionally be the same result as if TPS damage became more severe and would result in increased selection pressure from chemical pesticides and increased environmental impact. Chemical build-up in waterways because of agricultural run-off in rice is already a regulatory concern (Orlando and Kuivila, 2004). Prophylactic sprays made in situations where shrimp infestation is low or non-existent are, at best, an economic waste for the grower and, at worst, increases risk of resistance development. Pyrethroid resistance is already a growing concern for

this industry (Espino, *personal communications*; Grettenberger, *unpublished data*) and growers, in addition to lacking a wide pool of useful chemicals, lack the non-chemical control methods to reduce pyrethroid selection pressure (J. Bloese et al., 2024; Espino et al., 2024).

Understanding the fundamental biology and ecology of an agroecosystem is the first step in creating a successful IPM program. The development of TPS and rice have, until recently, been treated as separate components of this system and their interaction has not been clearly illustrated. Future studies that improve upon the current design may help to further parse out damage potential in an increasingly unstable climate. Of the metrics collected, root length during the study was not measured and was only recorded at the end. Since the root system is what makes the plants so vulnerable to TPS, a better understanding of their growth rate under different temperature conditions may help growers determine when they are at reduced risk of stand loss. We also did not collect data on TPS hatch rate, or explore other metrics of life history and fitness. Future studies should investigate TPS response to climate in greater detail, as changes to hatch rate or neonatal mortality may impact the severity of future infestations.

Ultimately, these studies could be considered preliminary work continued plant and pest interaction studies in this system. A lack of understanding of TPS life history and behaviors in rice will only make it more challenging to effectively control this pest. If we continue investigations into the plant and pest interactions, we will be able to more accurately predict and mitigate damage. Developing better action thresholds will help minimize damage, but better scouting methods must also be made available. Scouting methods that can detect TPS as early as possible should be developed to maximize a shortened window of management action. Alternative pest management strategies may also be useful in reducing reliance on pyrethroid insecticides, giving growers both a more complete set options to include in management plans

and reducing the risk of developing resistance. We must also consider the potential impact of climate change moving forward. Temperature and rainfall instability due to climate change will cause major disruptions for every crop grown in the Central Valley, but will be especially damaging to high water consumption crops such as rice. If we are to maintain high production and yield standards in California rice, the proposed improvements to IPM in this system will become even more necessary.

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Tables:

Table 7: Treatments for growth chamber experiment.

Temperature Treatment (°C)	Timing	Shrimp Density
23.8 (low)	3 days after flood	No shrimp control
26.7 (medium)	5 days after flood	1 shrimp
29.4 (high)	-	3 shrimp

Table 8: Treatments for greenhouse experiment.

Temperature Treatment (°C)	Shrimp Density	Average Achieved Temperature (°C)
23.8	No shrimp control	24.01
	3 shrimp	24.22
	5 shrimp	23.62
29.4	No shrimp control	28.30
	3 shrimp	28.82
	5 shrimp	28.56

Table 9: Average starting size of shrimp for greenhouse experiment.

Replicate	Number of shrimp	Average length (cm)
1	15	0.495
2	15	0.480
3	15	0.433
4	15	0.413

5	12	0.448
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Figures:

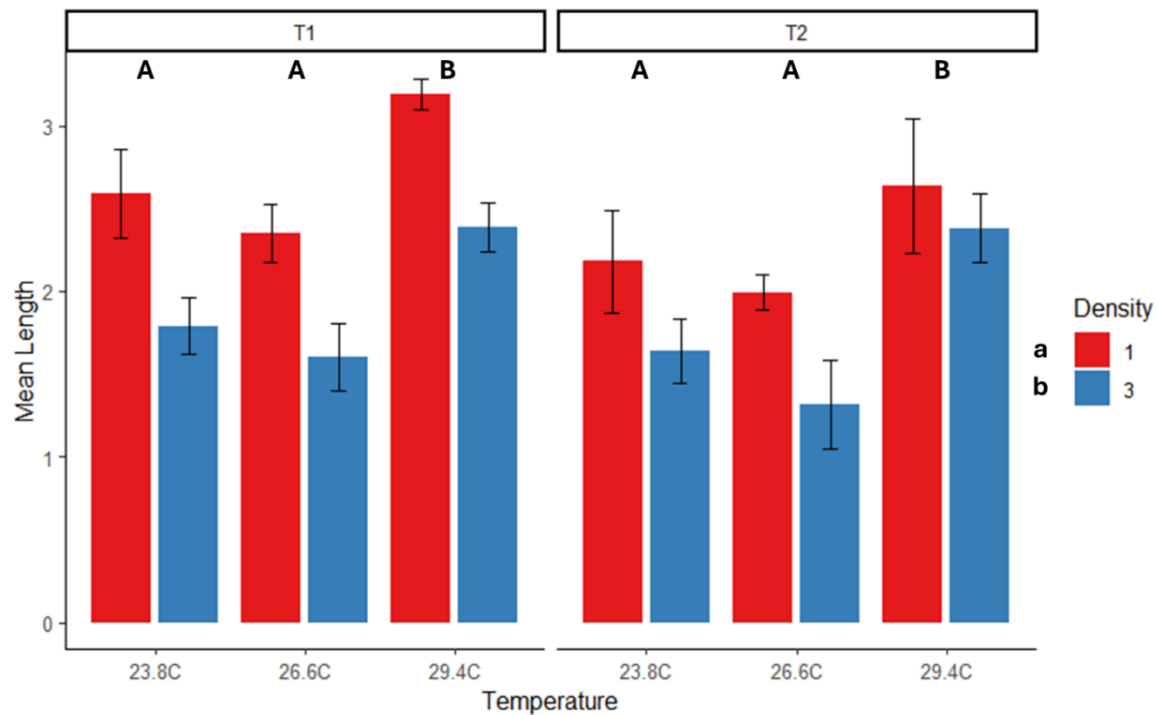


Figure 8: Final shrimp size at harvest for all temperatures, densities, and timings. Density refers to number of TPS. Length is measured from tip of carapace to end of tail excluding filaments. Final shrimp size at harvest for all temperatures, densities, and timings. Capital letters indicate significant differences ($\alpha = 0.05$) between temperatures; lowercase letters indicate significant differences between densities and are indicated on the legend.

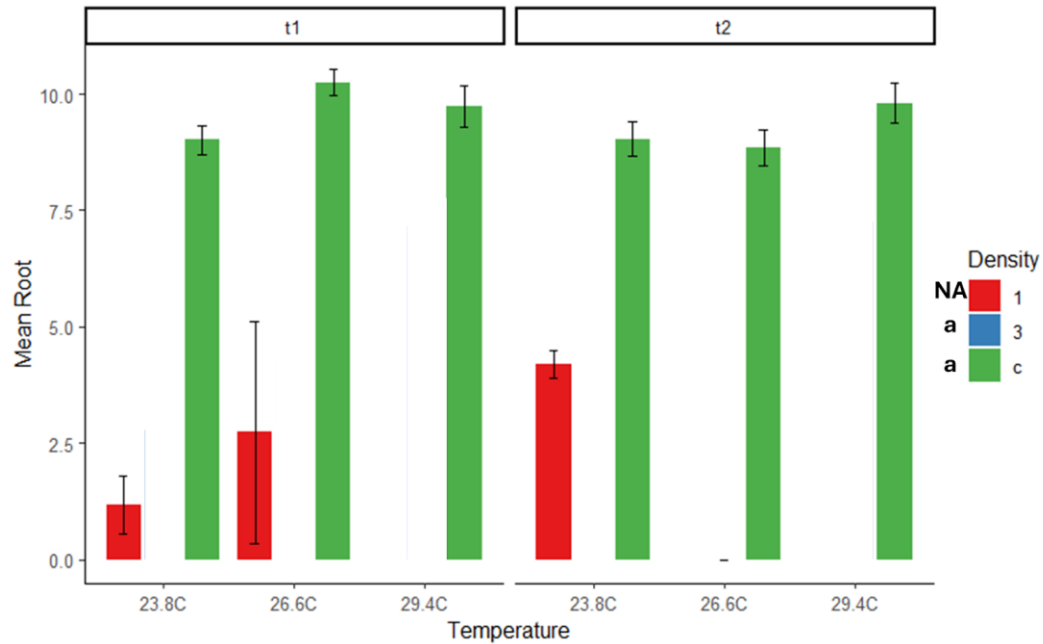


Figure 9: Plant root size at harvest in the growth chamber study. Density refers to number of TPS. “c” is shorthand for the zero-TPS control treatment. Root was measured from seed to end of longest root. Treatments which had no harvest data are removed from this plot and analysis. Letters indicate significant difference ($\alpha = 0.05$).

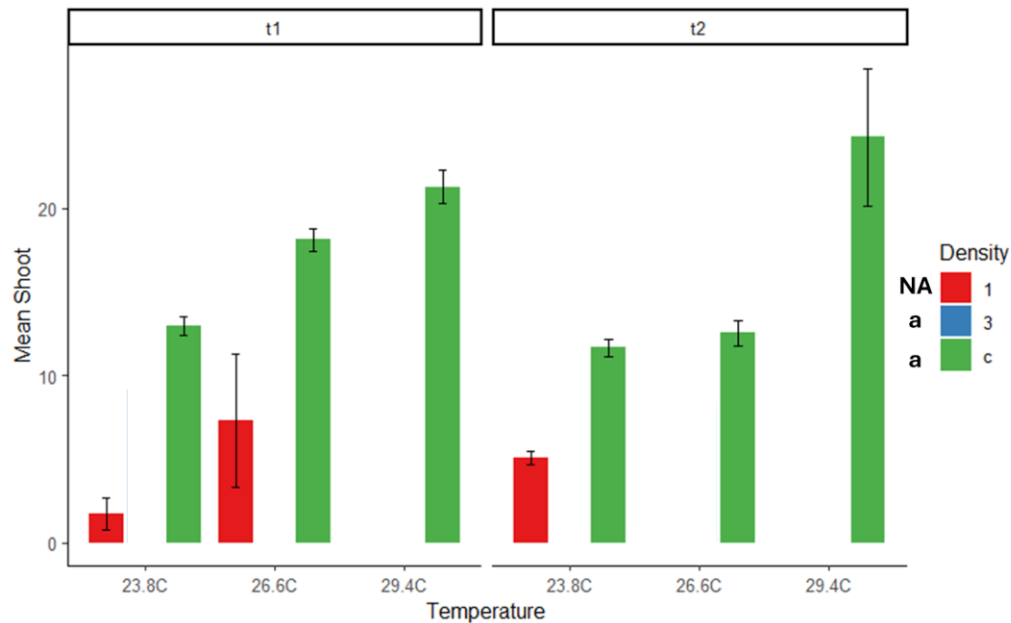


Figure 10: Plant shoot size at harvest in the growth chamber study. Density refers to number of TPS. “c” is shorthand for the zero-TPS control treatment. Shoot was measured from seed to end of longest leaf. Treatments which had no harvest data and cases where one or

more of the three shrimp in the 3 shrimp density treatment died and were not replaced are removed from this plot and analysis. Letters indicate significant difference ($\alpha = 0.05$).

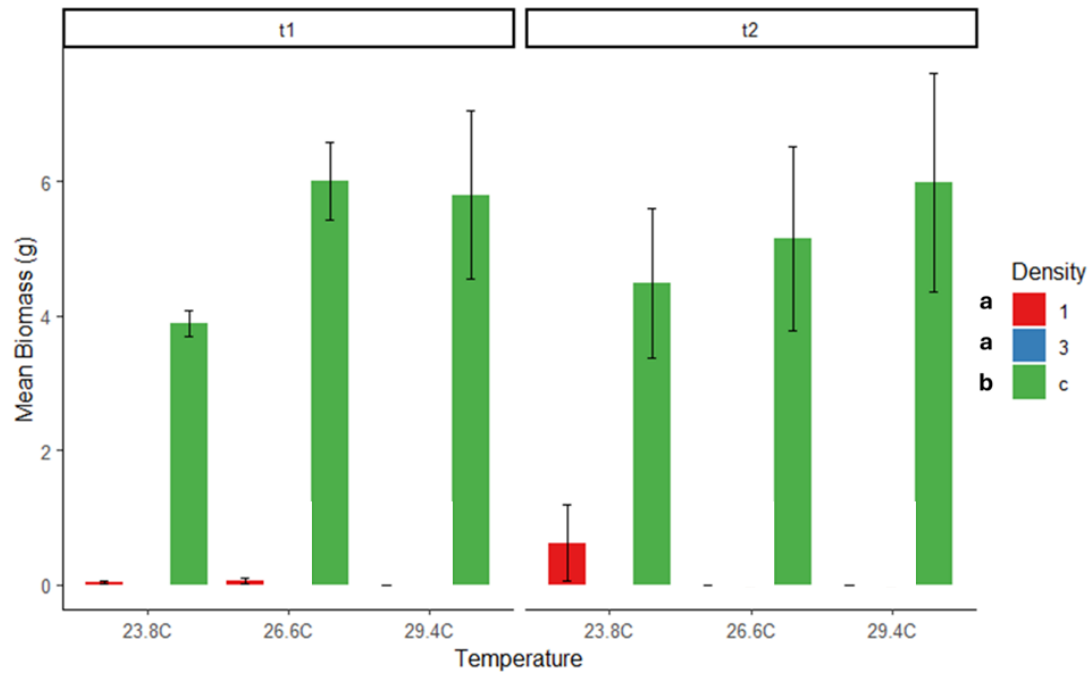


Figure 11: Average biomass at harvest in the growth chamber study. Density refers to number of TPS. “c” is shorthand for the zero TPS control treatment. Treatments are grouped by planting timing, indicated by T1 or T2. Treatments with no harvest data were recorded as having a biomass of 0. Cases where one or more of the three shrimp in the three-shrimp density treatment died and were not replaced are also excluded. Letters indicate significant difference ($\alpha = 0.05$) between treatments.

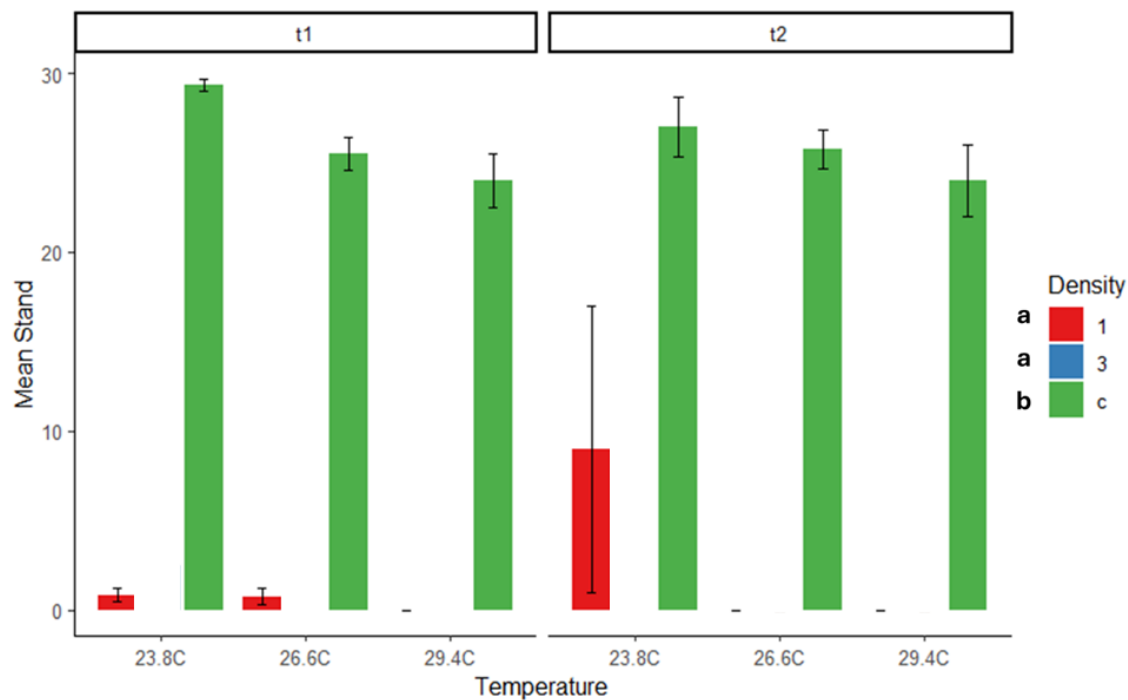


Figure 12: Average stand at harvest in the growth chamber study. Density refers to number of TPS. “c” is shorthand for the zero TPS control treatment. Treatments with no harvest data were recorded as having a stand of 0. Letters indicate significant difference ($\alpha = 0.05$).

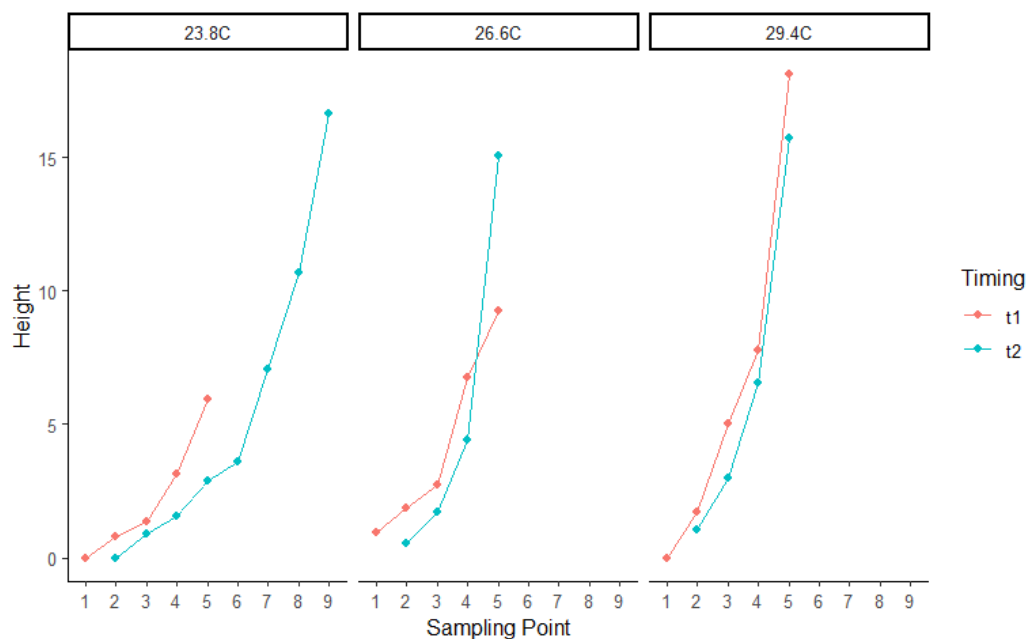


Figure 13: Average rice height at various sampling points. Average rice height at sampling points. Timing refers to planting timing. First sampling point was two days after Timing 1 (t1) was planted. Sampling for Timing 2 (t2) began at sampling point 2. Two reps in 23.8 °C treatment grew slower than other reps, resulting in later harvest. Line color indicates planting timing, and treatments are grouped by temperature.

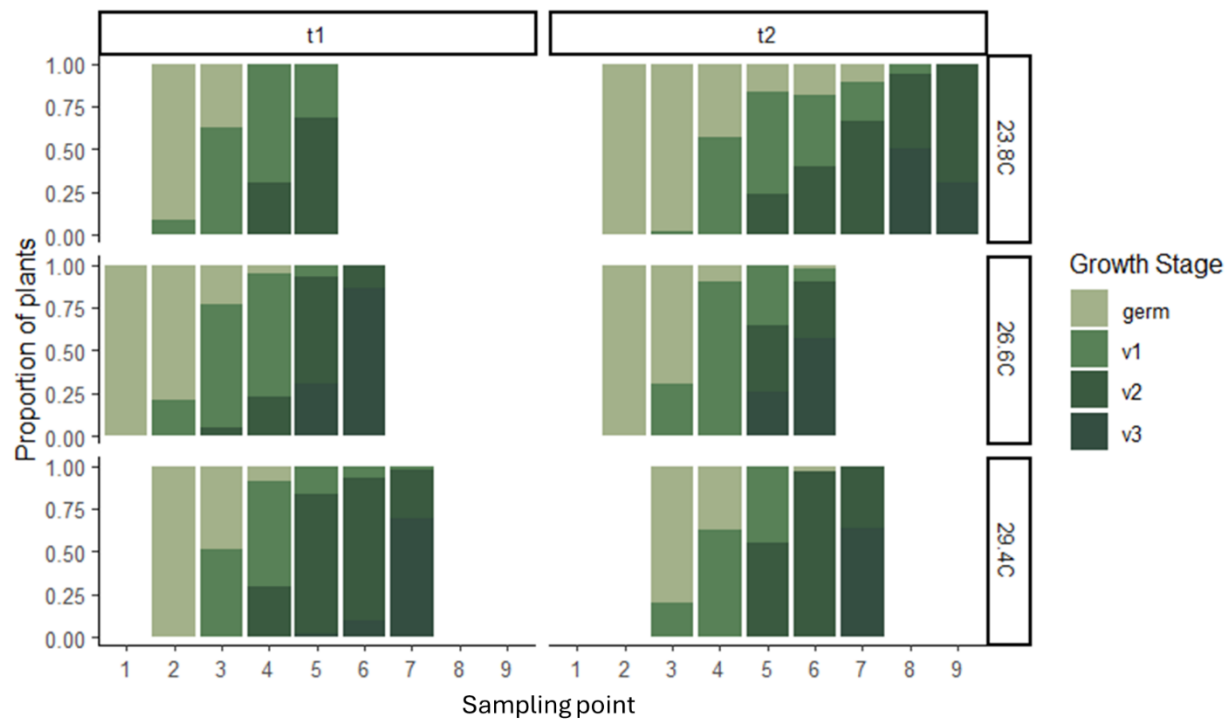


Figure 14: Proportion of germinated plants in different growth stages. First sampling point was 2 days after Timing 1 (t1) was planted. Sampling for Timing 2 (t2) began at sampling point 2. 2 reps in 23.8C treatment went longer than other reps. Growth stage at harvest not included for 23.4C treatment.

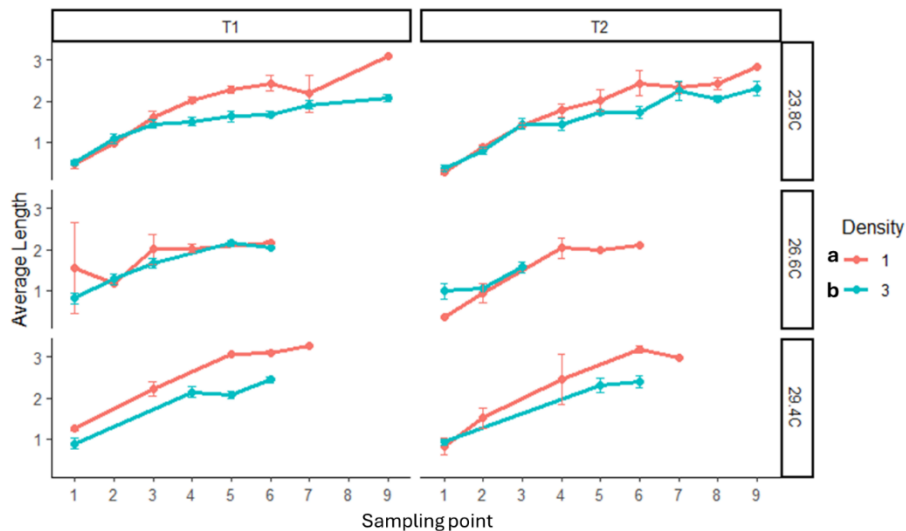


Figure 15: Average shrimp size over time. First sampling point was 2 days after Timing 1 (t1) was planted. Sampling for Timing 2 (t2) began at sampling point 2. Line color indicated density treatment. Letters indicate significant differences between treatments. Average length varied only by density, as indicated at the legend.

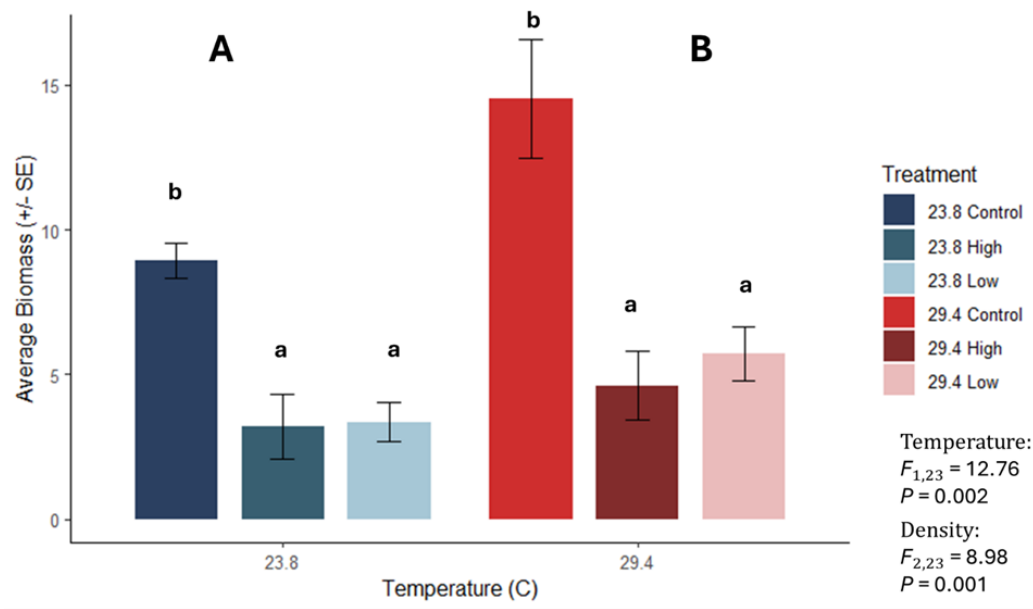


Figure 16: Mean ($\pm$ SE) biomass (g) of rice at harvest in greenhouse study. Results are grouped by temperature ($^{\circ}$ C). Treatment combinations are identified by temperature (23.8 or 29.4 $^{\circ}$ C) and tadpole shrimp (TPS) density (Control, high, or low). Capital letters indicate the difference between temperature treatments, lower case letters indicate differences in density treatments based on post hoc comparisons. Temperature had a significant effect on biomass, with plants in the 29.4 $^{\circ}$ C treatment having a higher biomass on average than plants in the 23.8 $^{\circ}$ C treatment. Biomass was also significantly affected by TPS density. The no-TPS control treatments in each temperature level had higher biomass on average than either TPS density treatment. The low and high density treatments did not differ from each other.

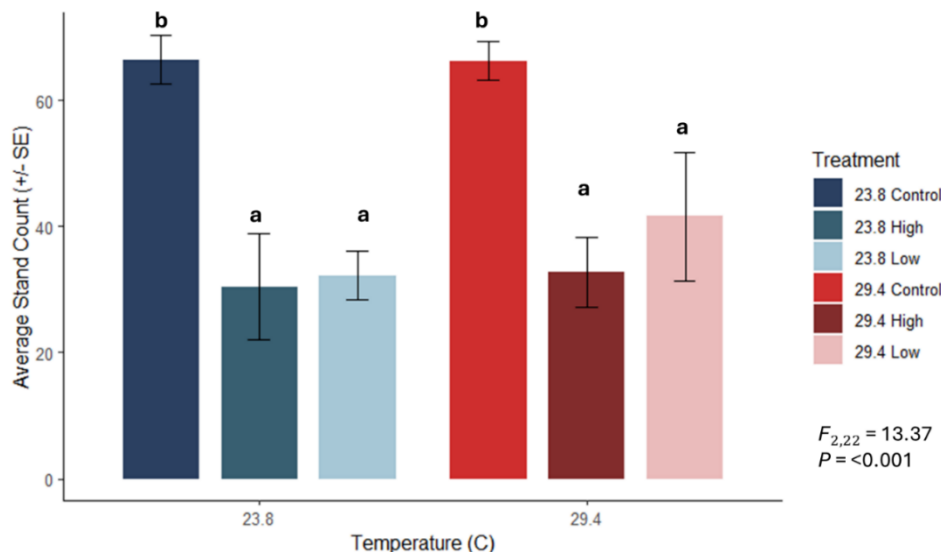


Figure 17: Mean ($\pm$ SE) rice stand count at harvest in greenhouse study. Results are grouped by temperature $^{\circ}$ C. Treatment combinations are identified by temperature (23.8 or 29.4 $^{\circ}$ C) and tadpole shrimp (TPS) density (Control, high, or low). Letters indicate significant differences between treatments. Differences were only found between shrimp treatments, not

temperature. No-TPS control treatments had a higher stand count on average than either TPS density treatment, which did not differ significantly.

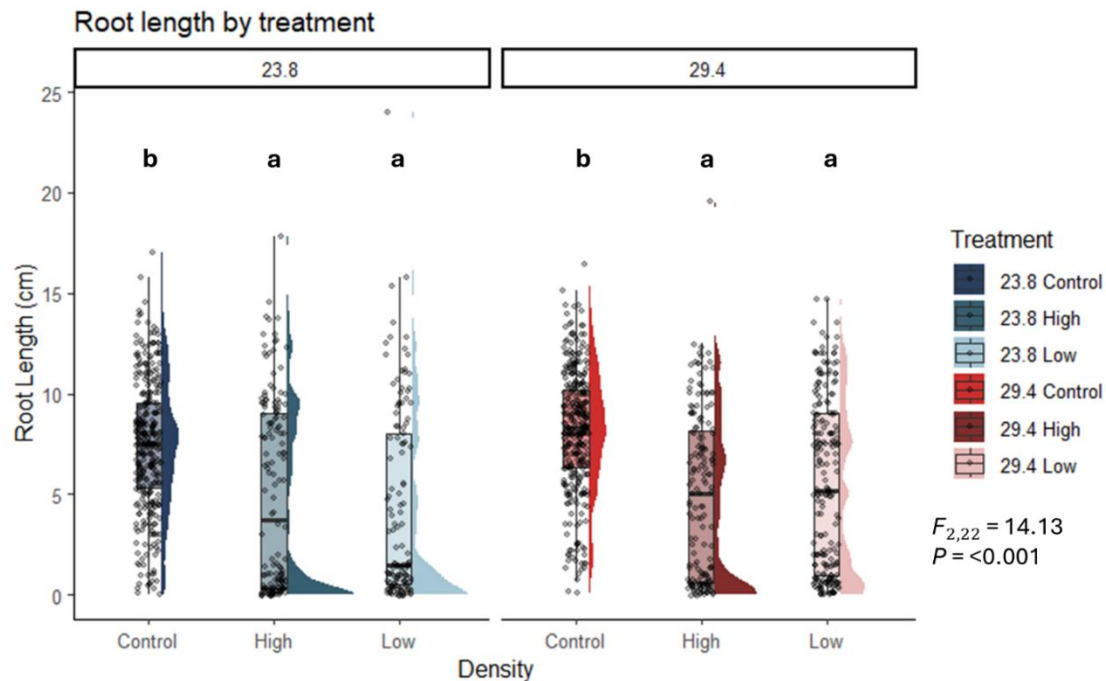


Figure 18: Raincloud plot showing rice root length (cm) at harvest in greenhouse study. Plots include boxplots, individual data points, and distribution of points on the right. Letters indicate results of pairwise comparisons, with values not sharing a letter significantly different at $\alpha = 0.05$.

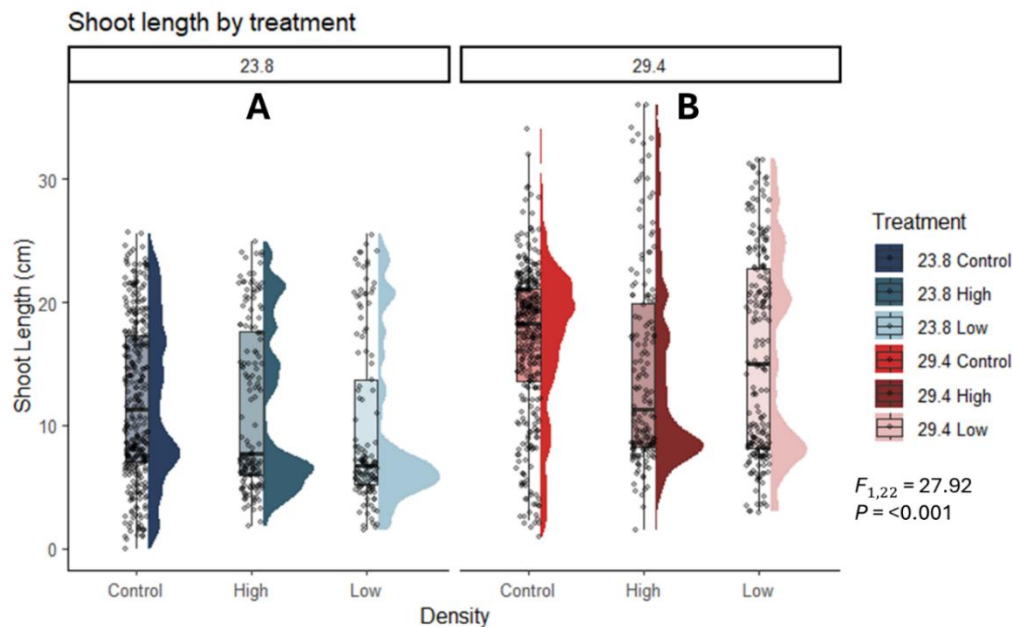


Figure 19: Raincloud plot showing rice shoot length (cm) at harvest in greenhouse study. Results separated by temperature and grouped by density. Plot includes boxplot, individual data

points, and distribution of points on the right. Capital letters indicate significant difference between temperature treatments.

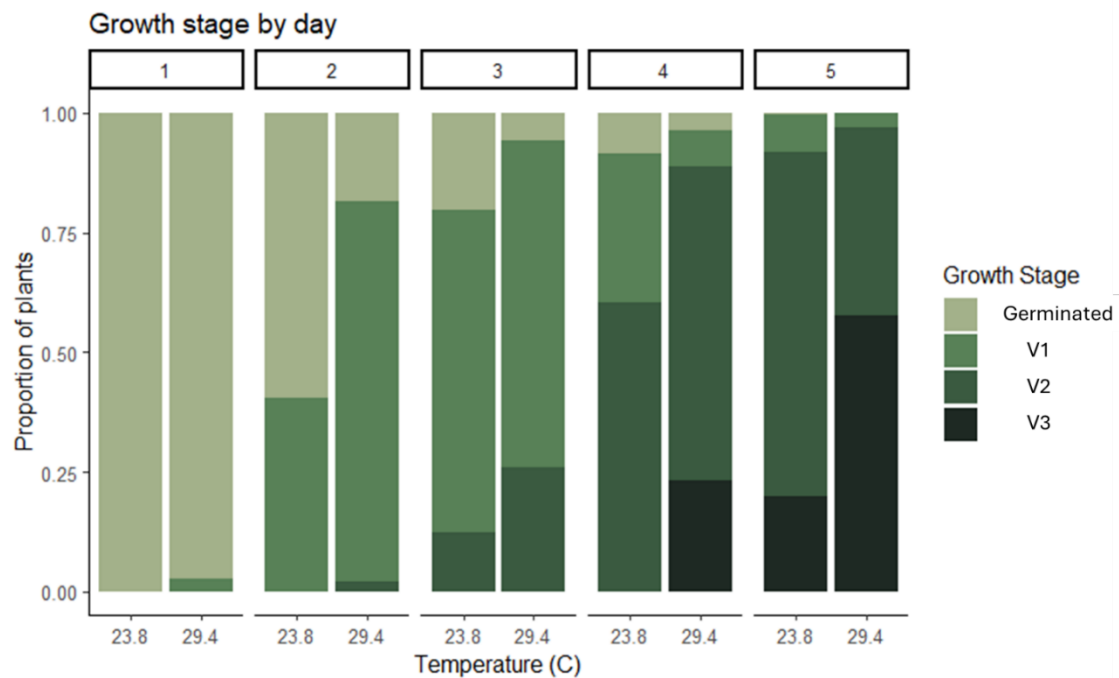


Figure 20: Growth stage proportion by sample day for the no-shrimp control treatments across the trial in greenhouse study. Results are separated by temperature treatment (°C).

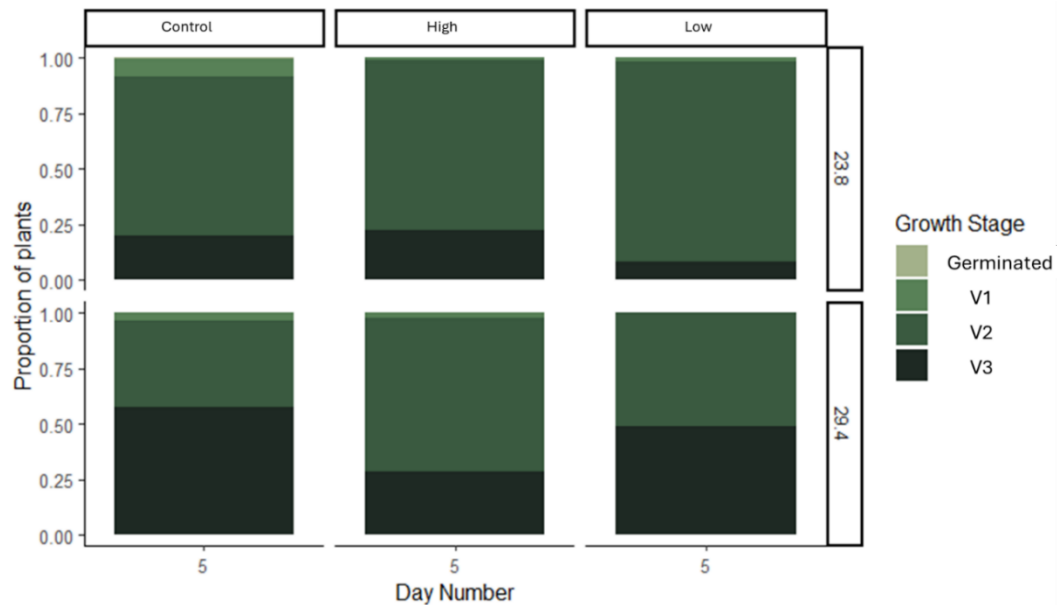


Figure 21: Growth stage proportion at harvest in the greenhouse study. Results are separated by temperature treatment (°C).

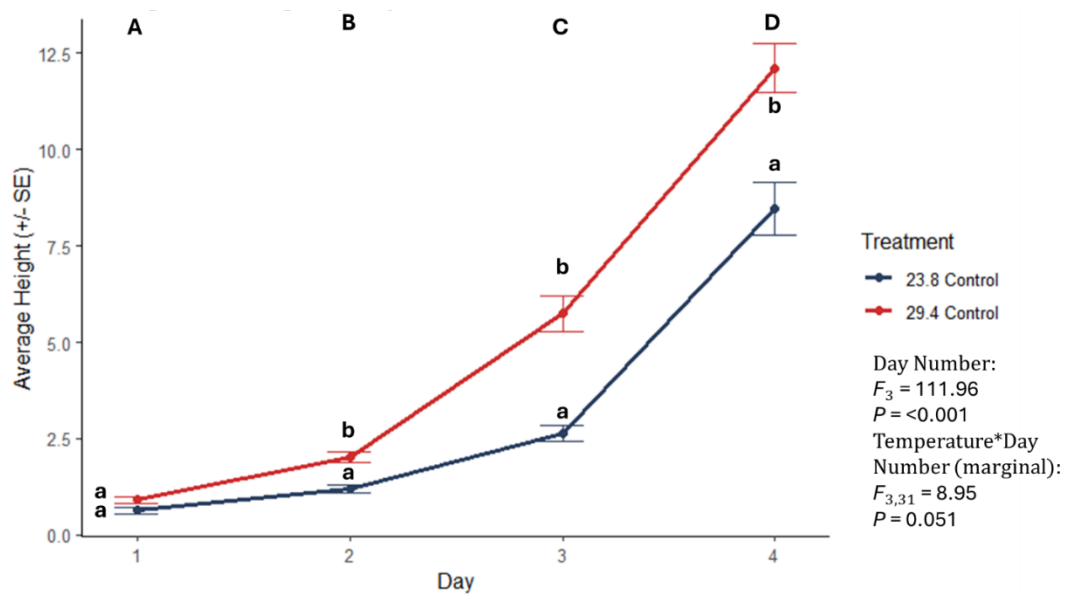


Figure 22: Average plant height by day in the control treatments excluding harvest. Control treatments were used as a proxy for treatments containing shrimp. The effect of day was significant and indicated by capital letters. The interaction between temperature and day was only marginally significant, but differences between temperatures on each day based on post hoc comparisons are still indicated by lowercase letter.

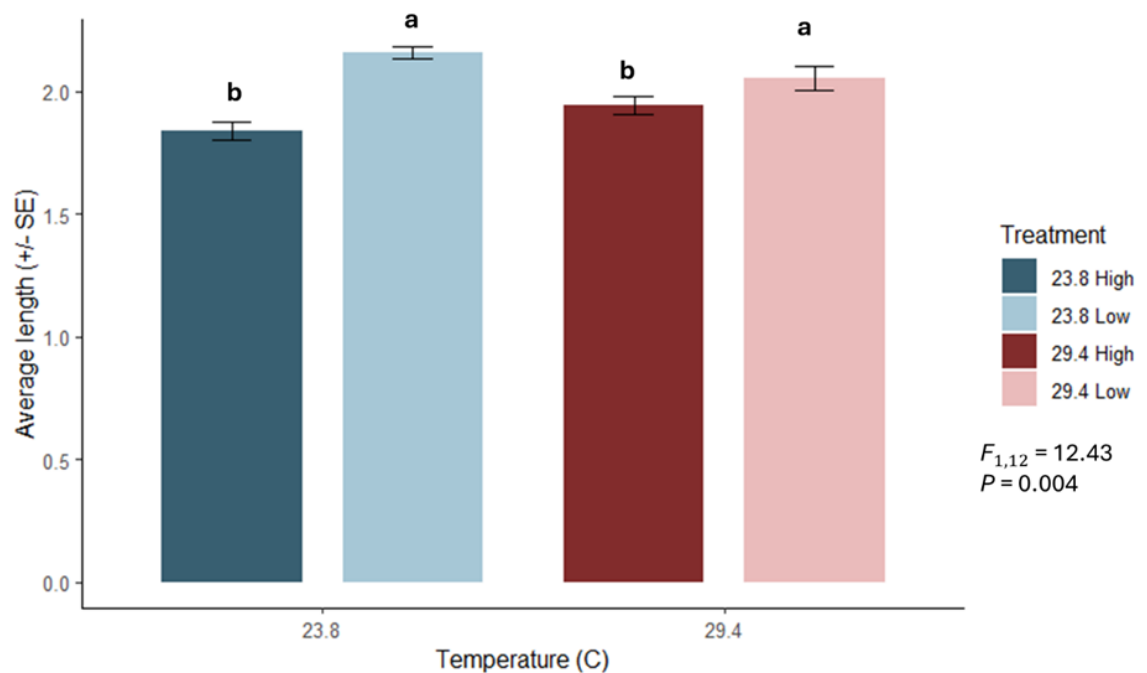


Figure 23: Average shrimp length at harvest in the greenhouse study. Letters indicate a significant effect of density. Shrimp in the low density treatment were on average larger than the shrimp in the high density treatment.

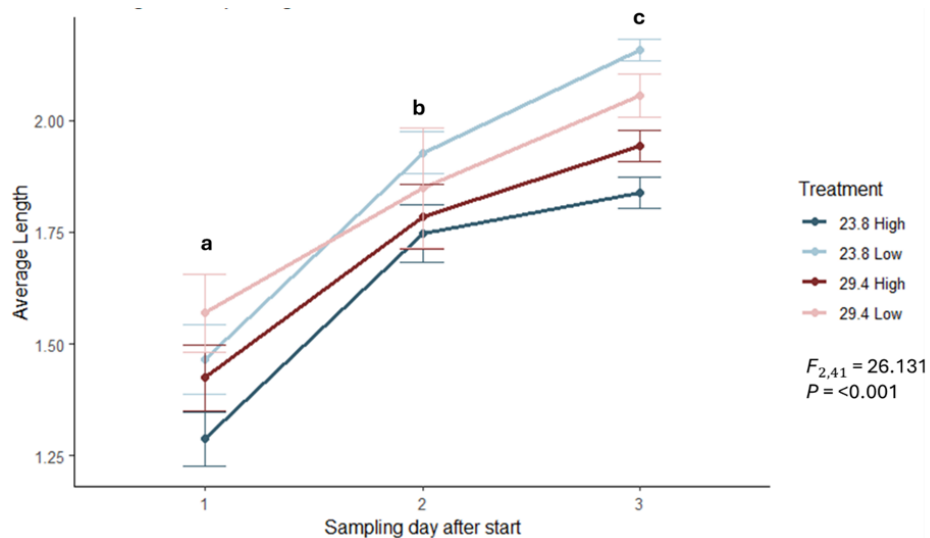


Figure 24: Average shrimp growth through trial for the greenhouse study. Letters indicate significant differences among factors. Excludes the starting size, but includes size at harvest. Shrimp length varied significantly by time.

All surf, no turf: Evaluating the mosquitofish (*Gambusia affinis*) as a potential biological control agent for tadpole shrimp (*Triops longicaudatus* (LeConte)) in California rice

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Key words: Biological control, rice, tadpole shrimp, mosquitofish, augmentative biocontrol

ABSTRACT

California rice growers are limited in their pest control options, and typically rely on pyrethroid insecticides for tadpole shrimp (*Triops longicaudatus* (LeConte)) management. Frequent use of chemical pesticides can lead to issues such as resistance and environmental contamination.

California rice is a system that would greatly benefit from the development of a biological control program to reduce chemical inputs and give growers additional tools. We attempted to fill this gap in rice pest management programs by evaluating mosquitofish (*Gambusia affinis*) as a biological control agent for tadpole shrimp. We conducted trials to test the density of fish and the application timing needed to achieve control using complete randomized block design. We also conducted a factorial design individual feeding assay to determine the feeding potential of different size fish. Through our density trial, we determined that the necessary number of fish to control tadpole shrimp is likely infeasible at production scale. Our remaining trials also demonstrated that fish need to be added early and with precise timing to the field to achieve optimal control. Ultimately, mosquitofish are not a suitable control agent for this system, but the lack of control options needs to be addressed to improve integrated pest management practices in this system.

Introduction:

The tadpole shrimp (*Triops longicaudatus* (LeConte); TPS) is a vernal pool crustacean that, in recent decades, has become a key, early-season pest of California rice (J. Bloese et al., 2024). Tadpole shrimp damage newly planted and developing rice seedlings by feeding directly on the plants and damaging the roots. They also often uproot seedlings, due in part to their digging in search of food and to the damage caused directly to the root system (multiple). They also have desiccation-resistant eggs that can remain dormant in dry soil until they are reintroduced to water and hatch within 24hrs (Scott & Grigarick, 1979; Takahashi, 1977). California rice is water-seeded, meaning that rice fields are flooded prior to planting, and then pre-germinated seeds are dropped by airplane into the field (Tindall & Fothergill, 2012). Simultaneously, as fields are flooding, TPS eggs stored in the soil bank begin to hatch, meaning the pest population begins to grow before the crop enters the field. Rice planted in this manner is especially vulnerable to TPS damage because the developing seedlings have weak root systems (Tindall & Fothergill, 2012). However, once the seedlings have grown tall enough to breach the surface of the water, their roots are more established, and they are generally considered safe from TPS damage (Espino et al., 2024). It takes approximately eight days for TPS to reach maturity and for rice to breach the surface of the water, but there is also often a delay of several days between flooding and planting (Scott & Grigarick, 1979; Takahashi, 1977; Tindall & Fothergill, 2012). This can create a developmental “arms race” between the TPS and the rice, increasing the damage potential. Severe TPS damage can result in reduced stand and yield, and muddy water can disrupt photosynthesis and development (Tindall & Fothergill, 2012). Currently, management tactics for this pest are limited to chemical applications, and addressing management in the classic integrated pest management (IPM) framework has room for improvement.

TPS management in California rice is difficult and is almost entirely reliant upon chemical control. However, due to TPS's tendency to forage in the mud at the bottom of basins, scouting and taking visual counts of TPS can be difficult and can be made more difficult with turbid water in windy conditions (Grigarick et al., 1961). Wind can create turbid conditions and TPS activity can also muddy the water, which can either mask the presence or make density estimation difficult. In the end, many will choose to prophylactically apply pyrethroids to control for TPS (J. B. Bloese et al., 2021). Conventional and organic rice production has relied on chemical insecticides for TPS management. In conventional systems, growers have historically used DDT, parathion, and copper sulfate, but those insecticides have since been replaced with primarily pyrethroids (Grigarick et al. 1985; Rosenberg, 1947). Organic production is even more restricted in terms of available chemistries and is essentially limited to copper sulfate. It is hypothesized, though, that with the banning of post-harvest burns in rice fields, copper sulfate may be less effective as it binds to the organic material left behind (Grigarick et al., 1961; J. Bloese et al., 2024; Spencer et al., 2006). Dependency on chemical insecticides can lead to widespread control failure, as has happened historically with parathion and has been reported recently with lambda-cyhalothrin (L. Espino, personal communications).

Biological control could be an option for TPS but is not currently relied upon for management of this pest. Rice fields are diverse ecosystems supporting over 20 arthropod taxa, though higher counts of individuals may be found in organic fields (Hesler & Grigarick, 1992). Natural enemies such as predatory beetles (for instance, *Hydrophilus triangularis*, *Laccophilus maculosus* and *Tropisternus lateralis*) are present in rice fields, but concerted efforts to use them as biological control agents are not regularly practiced, if at all (Hesler & Grigarick, 1992). These predatory beetles have been documented to feed on other pests of rice, namely rice seed

midge, but the extent to which they provide natural control of TPS is not known (Veneski and Washino, 1972; Zalom & Grigarick, 1980). Predatory beetles *Laccophilus maculosus* and *Tropisternus lateralis* were trialed as control agents for TPS, but these beetles' populations can be unpredictable and would need a concerted effort to augment the populations (J. Bloese et al., 2024). As it stands, neither conventional nor organic production have established protocols or practices for biological control, indicating a clear area for improvement of IPM in this system. This is especially true of organic production, which is far more limited in pest control tactics. As environmental and human health concerns become more prevalent, non-chemical alternative control measures could become more valuable (Hesler & Grigarick, 1992).

Augmentative biocontrol could work in the California rice system, but may face limitations. Augmentative biological control is the release of large numbers of reared control agents with the goal of either supporting existing populations or overwhelming pest species with natural enemies (Collier & Van Steenwyk, 2004). Augmentative biological control is often effective in short-season crops where enemy species are low in number and conservation biological control is ineffective. Augmentative control is often employed across systems such as orchards, field crops, and greenhouses (Van Lenteren, 2012a). The seasonal flooding and draining of fields, could make establishing recurring or self-sustaining populations of natural enemies more difficult, thus making this system a good candidate for augmentative releases. This technique can also be very successful in crops with relatively light pest infestations (Parrella et al., 1992). Augmentative biocontrol could be well suited for rice fields, particularly those with a history of low level TPS infestations. However, since TPS eggs do not hatch uniformly on a year-to-year basis, the success of an inundative release could vary from year to year. The window for TPS damage is very short, and at most spans the first 2-3 weeks of the growing

season. The pest and the natural enemy must also be temporally aligned to achieve maximum control potential. Given the short release window in rice, it is critical to select a natural enemy that can predate on TPS successfully in most if not all its life stages (Collier & Van Steenwyk, 2004) . There are a number of reasons that may cause augmentative biocontrol to fail, such as an unfavorable environment due to climate, enemy dispersal, and rearing and handling of the natural enemy (Collier & Van Steenwyk, 2004). Abiotic factors such as the economic impact of acquiring and releasing the natural enemy is also important, as the selected organism must be cost competitive to conventional methods to encourage program uptake. Understanding the system, pest, and potential risks for this method is necessary when selecting a natural enemy that can withstand such challenges and be successful.

Mosquitofish are already employed as control agents for aquatic mosquito larvae and are a prime candidate for augmentative biocontrol of TPS (Wheeler et al., 2022). The addition of this biocontrol agent into the vector management program has demonstratively reduced chemical treatments, inspection efforts, and control costs, begetting economic and environmental benefits (Lichtenberg, 1987). Mosquitofish are generalist predators, fairly hardy , and can be found in California's water ways (Cech et al., 1985; Walton, 2007), making them reasonable candidates as control agents for TPS. As generalist predators, mosquitofish have often been documented feeding on crustaceans such as copepods and zooplankton (Linden and Cech, 1990). It is reasonable to assume that they could predate on TPS, particularly in their neonatal forms and early mature stage. Mosquitofish, as visual hunters, typically perform better in open waters with sparse vegetation (Bence & Murdoch, 1986, Linden and Cech, 1990). Early season rice fields, when TPS are abundant and actively damaging rice, are sparsely vegetated even when planted due to the rice seedlings small size. The plants' small size may provide enough open water to

optimize the fishes' hunting capabilities. If the deployment of mosquitofish could be adapted by rice growers to suit the needs and timing for TPS control, IPM for rice production could be made more robust.

Mosquitofish have been tested in small scale trials as potential biocontrol agents for TPS with promising results (J. Bloese et al., 2024). Experimental arenas consisted of 1m² aluminum rings and fish were stocked at treatment rates of either 4 or 5 fish per 1m². In these conditions, mosquitofish were found to control TPS effectively, with no statistical difference in control compared to field rates of lambda-cyhalothrin (0.139 L/ha). Promising as the results are, there are still uncertainties surrounding the potential of mosquitofish. Some of these factors as identified by the authors are application timing and fish size and their predation abilities. Fish were applied uniformly two days after flooding in this study, but given the biology of TPS and the delay between flooding and planting and its relationship to damage potential, it is still unknown what the application window for effective control is. TPS control is also likely affected by fish size. Mosquitofish predation is correlated with fish size in that they will eat what they can successfully capture (Bence & Murdoch, 1986). TPS grow quickly, so understanding the predation capabilities in relation to size would be informative for augmentation success. These experiments demonstrated the potential of mosquitofish as a control agent, but were performed in the field at small scale. A larger scale experiment with greater variation in fish density application rates are needed to fully understand the potential of mosquitofish.

In this study, we conducted a series of field trials to evaluate the potential of mosquitofish as a biological control agent for TPS. This study had three primary objectives: Objective 1) to test what density of fish needs to be deployed; Objective 2) to test the optimal application timing; and Objective 3) to run fish feeding assays to assess the feeding potential of individual fish.

Materials and Methods:

We conducted three different field experiments to assess mosquitofish as a potential biocontrol agent. Two separate field trials were conducted to evaluate the necessary densities of fish and the optimal application timing. A third trial evaluated the feeding potential of individual fish. All experiments were conducted at the Rice Experiment Station in Biggs, California. The fish density trials are referred to as Trials A, B, and C hereto forth. The fish application timing trial is referred to as Trial D. Refer to **Table 10** for full naming conventions, year, and field information.

Fish Density Trials

To test the effect of fish density on TPS populations, we used a completely randomized block design. We set up 3.1x3.1m square plots with 0.9m buffer zones between each plot in the field prior to flooding. The ring plots were made with 24in metal flashing with the bottom 15cm of the walls were bermed with dirt from the field. Water was able to permeate during flood, enabling shrimp to hatch within the plots, but the berms prevented TPS and mosquitofish from escaping once placed. The plots were planted with pre-germinated rice seeds, dispersed by hand at a rate of 150lb/ac two days after fields were flooded.

We assessed TPS counts every other day from the deployment of the trial until the rice was out of the water and no longer under threat from the shrimp (about 2 weeks from planting). To assess daily shrimp count, we used a 16in diameter PVC pipe placed directly into the mud to create a standardized sampling area. A 12x9.5cm aquarium net was used in a figure-8 fashion 10 times to scoop shrimp. In Trials A and B, sampling was conducted in the lower left and upper

right corners. In Trial C, all 4 corners of the plot were sampled. Tadpole shrimp counts from the corner samples were pooled as an estimate of total abundance for each sampling point. Field plot design can be seen in **Figure 25**. Up to five TPS were also collected from each of the plots and measured lengthwise from the tip of the carapace to the end of the tail, excluding the filaments, and widthwise across the widest part of the carapace. Seedlings were collected from the plots during the trials and measured for root and leaf shoot length. Seedlings collected from control plots were carefully pulled directly from the mud to get full root and shoot length. Floating seedlings from other treatment plots were collected and measured for damage assessment.

We tested five treatments of fish density. In Trials A and B, we used 0 fish, 10 fish, 25 fish, 40 fish, and insecticide plus 40 fish. In Trial C, the treatments remained the same except the insecticide control treatment did not use fish in addition to the insecticide. For the insecticide controls, diflubenzuron was used at a rate of 8 fl oz per acre. Diflubenzuron is a fish-safe insecticide used to prevent fish mortality in the event of leaks. We ran 5 reps of each treatment. All treatments were added at the time of planting, which was two days after the field was flooded. Sampling began Two days after.

Fish Application Timing Trial

To test the effect different application timings had on TPS populations, we established plots using the same methods described above in the fish density trial. Shrimp count data was also collected in the same way, summing counts across four corners for each plot. Seedling data was also collected identically. We tested 4 timing treatments: at planting, 1 day after planting (DAP), 3 DAP, with an additional insecticide treatment added at planting. This trial also used

diflufenzuron applied at a rate of 8 fl oz per acre. We ran 5 reps of each treatment, and all treatments used the same, high density of 40 fish. Planting occurred two days after flood.

This trial was likely affected by drift from an herbicide application made to an adjacent field. The herbicide's active ingredient was thiobencarb, which is highly toxic to shrimp (Valent U.S.A. LLC, 2021). The adjacent field was treated prior to experimental setup, when the field was prepped but not yet flooded. The application timing trial still had measurable shrimp counts, despite the drift. It is possible that, due to the construction methods, the herbicide was able to bind to the plastic liner instead of floating freely in the water or lingering in the soil. Thus, the plots were potentially "filtered" by the plastic used to make the walls and limited the exposure to the shrimp.

Fish Feeding Assay:

To test the compatibility of various fish sizes and shrimp sizes to determine their feeding capability, we conducted a feeding assay in the field. We created size bins for both fish and shrimp. Fish were classed as either "small" if they were shorter from nose to base of the tail (excluding fin) than 1.5cm, and "large" if they exceeded that benchmark. Shrimp were divided into small, medium, and large. Shrimp were measured from the tip of the carapace to the end of the tail, excluding the filament. Shrimp up to 0.5cm were deemed small, medium ranged from 0.6cm to 1cm, and large exceeded 1.1cm. Sizes of both species were chosen somewhat arbitrarily, but fish size was more or less determined by maximum size and biological differences, whereas shrimp size was meant to be a proxy for time. Shrimp were hatched in a greenhouse and brought to the field to ensure that there were sufficient numbers of each size. Shrimp grow rapidly, and eggs were flooded on a schedule to get the desired size. Small shrimp

were flooded 4 days before trial, mediums 5-6 days before, and large shrimp were flooded at least 7 days before the trial.

This experiment was run in 2024 and 2025 in field J7. We used mesh office waste baskets 34 cm tall with a top diameter of 28 cm and bottom diameter of 23 cm as the assay arena. A five-gallon paint strainer bag was used to exclude other organisms from the arena, and an internal frame of aluminum window screening, 18cm tall, was inserted to reinforce the mesh bag and maintain the full inner area of the arena. Arenas were pressed into the mud for stability so that the depth of the water from surface to floor was equal to that of the rice field, which was about 4in. We used a factorial design to pair each size of shrimp with either a small, large, or no-fish control to assess background mortality. Treatments were randomized within each rep. One fish was placed in each arena, except in the no-fish controls. Five shrimp were paired with the fish in the small and medium shrimp treatments. In the large shrimp treatments, only three shrimp were placed in the arenas due to cannibalism concerns in a confined space. The assays ran overnight and were evaluated approximately 24 hours after starting. Missing shrimp are assumed and regarded as dead. Remaining shrimp were recorded and subtracted from the total to calculate the percent mortality.

Data analysis:

We analyzed data using R version 2022.12.0+353. We analyzed overall shrimp density data with linear mixed effect models using the lme4 package (Bates et al., 2015). Fixed effects included treatments and block. Tukey's HSD was performed for post hoc analysis, and estimated marginal means were calculated using the emmeans package (Lenth 2023) to determine treatment effects.

To analyze shrimp density over time, we averaged daily shrimp counts and used linear mixed regression. Fixed effects included the interaction between treatment and date, block, and a random effect of ring to account for repeated measurements. In Trials A and B, when seedling data was taken twice, the mean length of each day was analyzed using linear mixed regression, with the interaction of treatment and date as a fixed effect, and ring as a random effect. When sampled only once in Trial C, the only fixed effect was treatment. Otherwise, the model was the same and both analyses used estimated marginal means to do pairwise comparisons. Yield data was analyzed with linear mixed regression, with treatment as a fixed effect and block as a random effect. We ran a Type III ANOVA using Satterthwaite's method for post hoc analysis. Stand data was analyzed with linear regression with treatment and block as fixed effects. Shrimp size was analyzed using the mean length of shrimp collected, in a linear regression model. Treatment and block were the fixed effects. Estimated marginal means and Tukey's HSD were used for post hoc analysis. Data analysis methods were the same for both density and application timing trials. Feeding assay data was analyzed using a linear model. The mortality proportion was analyzed as a response of the interaction between fixed effects fish size and shrimp size. Differences between treatments were analyzed using estimated marginal means with Tukey's method.

Results:

Density Trials:

Overall shrimp count: In Trial A, we saw differences in the efficacy of fish density on shrimp density ($F_{4,16} = 16.86$, $P < 0.001$; **Figure 26**). The treatments had a mixed effect on shrimp

density. The 0 fish treatment had significantly higher shrimp counts than any of the treated plots. Of the fish treatments, the 10 fish treatment performed the worst, while the 25 fish and 40 fish densities performed similarly to the insecticide control. In Trial B, we saw an effect of treatment on shrimp density ($F_{4,16} = 4.53$, $P = 0.002$; **Figure 26**). The insecticide control treatment performed best and achieved the highest level of control, whereas our negative control, 10 fish and 25 fish treatments achieved the lowest levels of control. These treatments did not differ significantly. The 40 fish treatment performed slightly better, achieving a level of control between the other fish treatments and the insecticide control. In Trial C, we found that treatment had no effect on the overall average density of shrimp ($F_{4,16} = 1.71$, $P = 0.20$; **Figure 25**). TPS were still present in the insecticide control, implying a lack of control from the insecticide.

Shrimp over time: In Trial B, the interaction of treatment and date was highly significant ($F_{16,80} = 2.63$, $P = 0.002$; **Figure 27**). The first and second sampling dates were not significantly different from each other, but were significantly different from our third, fourth, and fifth dates. In Trial A, we detected a slight significance in the effect of dates ($F_{4,80} = 2.44$, $P = 0.053$; **Figure 27**). The first and third sampling dates were most different from each other, with our second, fourth and fifth sampling dates showing a mixed level of effect. In Trial C significant differences were also shown between dates ($F_{4,80} = 21.936$, $P < 0.001$; **Figure 27**). The first sampling date was most different from all the other dates, The second date was similar to the first and third date, and the fourth and fifth date were similar to each other but different from the rest.

Seedling root length: In Trials A and B, we collected seedling data twice. In Trial A, we found differences in seedling root measurements by treatment ($F_{4,40} = 12.58$, $P < 0.001$; **Figure 28**). We saw an effect of interaction between treatment and date ($F_{4,40} = 3.39$, $P = 0.03$). The insecticide control treatments differed from each other between sampling dates ($P = 0.001$), but

all other treatments showed no difference. In Trial B, we did see differences between treatments ($F_{4,41} = 18.86$, $P < 0.001$; **Figure 28**) and between each sampling date ($F_{1,41} = 11.42$, $P = 0.002$), but did not see an effect of interaction between treatment and sampling date ($F_{4,41} = 0.55$, $P = 0.70$). On each sampling date, only our insecticide control plots showed differences from the other treatments. The 25 fish treatment was the only treatment to show differences across the sampling dates ($P = 0.01$). In Trial C, we only collected seedling data once. We saw the effect of treatments ($F_{4,16} = 27.14$, $P > 0.001$; **Figure 28**), and of rep ($F_{4,16} = 3.58$, $P = 0.03$). Plants collected from our insecticide control treatments were significantly different from the other treatments. These plants had longer, complete roots as opposed to the other treatments, that incurred damage from shrimp. The 0, 10, 25, and 40 fish treatments did not differ significantly from each other.

Yield and stand: We collected yield data at the end of the growing season to see the effect of mosquitofish control at the end of the growing season. Ultimately, none of the three trials in either year showed significant differences between treatments (A: $F_{4,21} = 1.19$, $P = 0.353$, B: $F_{4,21} = 1.22$, $P = 0.332$, C: $F_{4,21} = 2.08$, $P = 0.132$; **Figure 29**). In Trial C, we collected stand data as well, and saw an effect of treatments ($F_{4,21} = 4.77$, $P = 0.010$; **Figure 30**). We found that the insecticide control treatment had a stand count significantly different from the 10 and 40 fish treatments, which did not differ from each other. The 0 fish and 25 fish treatments did not differ from each other, and showed an intermediary effect on stand.

Shrimp size: Shrimp were collected in Trial C. There were no significant differences between treatments in length ($F_{3,14} = 0.99$, $P = 0.427$; **Figure 31**) or width ($F_{3,14} = 0.95$, $P = 0.444$; **Figure 32**).

Timing Trial:

Overall shrimp count: We ran a trial testing fish application timing and its effect on overall tadpole shrimp density. We saw an effect of treatment on overall TPS density ($F_{3,17} = 8.20$, $P = 0.003$; **Figure 33**). We found that the insecticide control treatment differed significantly from our 1 day after planting application. The planting application and 3 DAP treatment were similar to each other and showed intermediary effects of treatment on overall density. The planting application was not significantly different from our insecticide control, and the 3 DAP treatment was not significantly different from the 1 DAP treatment.

Shrimp over time: We found that only date had a significant effect on daily shrimp counts ($F_{5,79} = 10.22$, $P < 0.001$; **Figure 34**). Estimated marginal means were calculated and used for pairwise comparisons of treatments within each date. On the first sampling date, the planting application treatment and our 1 DAP treatment were significantly different, with the 3 DAP treatment showing intermediary effects. For the following sampling dates, the treatments did not vary significantly.

Seedling root length: We saw a difference in treatment effect on seedling root length ($F_{3,16} = 23.66$, $P < 0.001$; **Figure 35**). The insecticide control treatment was the only significantly different treatment, and the fish application treatments at planting, 1 DAP, and 3 DAP did not differ from each other.

Yield and stand: We took yield and stand count at harvest, and in both cases, saw no differences amongst treatments (Yield: $F_{3,16} = 1.92$, $P = 0.180$; **Figure 36**; Stand: $F_{3,16} = 0.27$, $P = 0.846$; **Figure 37**).

Shrimp size: Shrimp were not collected from this trial given low numbers at the start due to herbicide interference.

Fish Feeding Assay:

Shrimp mortality: There was a significant interaction between fish and shrimp size ($F_{4,83} = 4.54$, $P = 0.002$; **Figure 38**). Estimated marginal means were calculated and used to find the differences between shrimp treatments within fish sizes. In our no-fish controls, we saw no differences between mortality in shrimp size. In both fish treatments, we saw that fish of either size consumed statistically similar amounts of small and medium shrimp, and that large sized shrimp consumption was significantly different. On average, large fish consumed 86.67% of small shrimp and 68% of medium shrimp, whereas small fish only ate 62.22% and 54.55% respectively. Control mortality of small, medium and large shrimp was 20%, 27.27%, and 21.21% respectively.

Discussion

Ultimately, mosquitofish seem poorly suited as control agents in this system. We examined how the density of fish released and the release timing affected TPS numbers in large plot field trials. Our fish density trials each showed different levels of TPS control, but only achieved control comparable to insecticides at high density. Our application timing trial had some limitations, but it suggested that the best level of control is achieved if fish are released at planting. Meanwhile, our feeding assay corroborated those results by demonstrating that the fish were better at feeding on small and medium sized, and therefore younger, TPS. The need for early and precise timing of release further limits the potential of mosquitofish by imposing difficult, logistical challenges on growers.

Fish density had varied effects on tadpole shrimp presence but ultimately do not seem to effectively control TPS well in rice production systems. Only in Trial A did mosquitofish demonstrate real potential as a viable biological control agent. Even then, the fish treatments showed very little difference in efficacy, though higher densities of fish trended closer to the insecticide control treatment. In Trial C, the fish treatments did not affect overall shrimp populations (**Figure 26**). The mosquitofish had no effect on damage incurred by tadpole shrimp. Across all three trials, we effectively saw no difference of treatment in any of our damage metrics. We used root length of floating seedlings to assess damage during the period of TPS activity when fish are providing control. At all 5 sampling points across 2 years, we ultimately saw no significant differences between the fish treatments. All 5 trials showed that the fish treatments were only different from the insecticide control treatments. The initial collection point in Trial A showed seedlings in the 10 and 25 fish densities with slightly longer roots (Figure 28), but that effect was not visible in the second collection point. There was minimal influence of our

treatments on yield in all trials. 2023 was the only year in which stand count data was available and, despite variable stand counts, yield was consistent and did not differ across treatments (Figure 30; Figure 29). Though the fish did offer TPS control to varying degrees in Trials A and B, the fish do not provide control sufficient to reduce damage during the early season.

Production logistics could also limit the viability of mosquitofish as a control agent even if they provided control at higher densities. In Sacramento and Yolo Counties, the Sac-Yolo Mosquito Vector Control District (VCD) produces an annual average of 1632kg of mosquitofish, 75% of which get distributed to rice fields (Wheeler et al., 2022). Mosquitofish for vector control are typically stocked in rice fields at a rate of 0.22-0.67 kg/hectare with a kilogram of fish containing between 1300 and 2900 fish (Wheeler et al., 2022). Were we to extrapolate our density of 40 fish per 100sqft to a hectare, that would require over 43,000 fish to be added right at planting, assuming that occurred no more than 3 days after flood. Though the Sac-Yolo VCD could produce up to 4.7 million fish a year, their entire production would only be able to supply 110 hectares with the necessary density of mosquitofish. There are over 200,000 hectares of land devoted to rice production in California (University of California, Davis, n.d.). Relying solely on a single VCD to supply fish would make this strategy unfeasible at scale, and would require additional infrastructure to suit the needs for the larger rice production zone.

How quickly the fish are released appears important for the success of mosquitofish as biocontrol and we achieved the highest levels of control when fish were applied at planting when TPS are small. In Trial D, our release timing trial, fish applied at planting performed most similarly to the insecticide control. This trial was impacted by outside factors, which likely affected our results. Our randomized complete block design resulted in three out of five reps of the 1 day after planting application were arranged next to each other on the same side of the

field, and furthest away from the field treated with herbicide. Those three treatments may have had higher initial shrimp survival than those plots closer to the exposure point. Thus, our data likely reflect lower initial shrimp counts across all other treatments as opposed to poor fish performance in the 1 DAP treatment (Figure 33). Because of the herbicide's impact, it is difficult to draw strong conclusions from this trial. Our fish feeding assay, however, had more conclusive results. We found that both sizes of fish had relatively high feeding success on small to midsize TPS, but struggled to control large shrimp. Both small and large fish were able to achieve well over 50% mortality with small shrimp, but mortality dropped off steeply with increase in shrimp size and age. Small fish's predation dropped by 13% as shrimp increased from small to medium size, and large fish's ability dropped 24% (Figure 38). Given that size can be used somewhat as a proxy for TPS age, the fish are better at controlling younger shrimp as opposed to the fully mature individuals. Though this experiment was not designed to pinpoint the ideal number of days after flood to add the fish, earlier application could result in better shrimp control at a field scale. Mosquitofish feeding capabilities are linked to the size of the prey and the size of the fish itself (Wartsbaugh et al. 1980; Bence & Murdoch, 1986). The results of this assay do not necessarily corroborate the results of our timing trial but does add further evidence to support the hypothesis that an earlier application date will provide better control.

The predator release window for rice is short, and success of augmentation would depend on early release. Rice is generally considered safe from TPS damage once it breaches the surface of the water, giving the rice plants approximately 8-10 days to breach (Grigarick et al. 1960; Tindall & Fothergill, 2012; Weeks, 1990). This period assumes that the rice must breach before the shrimp are fully mature at about eight days post-flood, but the TPS can hatch and reach an economically injurious size by the time rice is planted (Scott & Grigarick, 1979; Tindall &

Fothergill, 2012). Fields can take multiple days to flood (Rutger & Brandon, 1981), and this delay can complicate the timing aspect significantly. A field that takes three days to fully flood may already have TPS neonates in significant numbers and further delay in predator release increases the potential for damage. Early release has been shown to provide greater pest suppression in some systems (Costamagna et al., 2015). Early presence of predators showed higher suppression of melon aphid than late presence, and the extended early and late presence of predators increased predation rates further. Late predation in this system is not as effective, likely because aphid populations had multiplied beyond the threshold of achieving control (Costamagna et al., 2015). Pest and crop phenology are also necessary considerations to optimize predation. Pearl millet is somewhat similar to rice in that the eggs of the millet head miner (*Heliocheilus albipunctella*), a pest species, are extremely difficult to scout for. Thus an understanding of this pest's phenology in relation to the crop and its parasitoid is crucial for augmentative biocontrol success. It was found that optimal release timing of the parasitoid *Habrobracon hebetor* is at panicle emergence or flowering because it takes up to two weeks for *Habrobracon hebetor* to establish, which coincides with the preferred larval instar stage for parasitization (Amadou et al., 2019). *H. hebertor* is not technically being released early, but it is being released ahead of the vulnerable stage. Giving it an establishment period reduces the numbers needed at initial release. If a control agent needs time to establish, a higher release rate may be needed to compensate for delayed releases. Ironically, in some cases of augmentative biocontrol, release rates mattered less than release timing and methodology (Crowder, 2007). Higher variegated grape leafhopper control was demonstrated (*Erythroneura variabilis*) with fewer green lacewings (*Chrysoperla spp.*) when release timing was synchronized with leafhopper egg

hatch as opposed to nymph densities (Daane et al., 1993). For control of TPS, near immediate releases would be necessary to target the small, vulnerable stage of the TPS to achieve control.

Furthermore, temperature in the early season may alter the release window and the efficacy of fish as a biocontrol agent. Anecdotally, we observed the detrimental effects of extreme weather events on biocontrol releases in this system. Each year's trials were conducted later than the previous year. Start dates for Trial C were nearly two weeks later than in the previous year. It is possible that a later flood date could coincide with warmer temperatures, which may have an effect on tadpole shrimp development (Atkinson, 1995). We attempted to run additional fish density and application timing trials in 2024, however each flood coincided with separate heat waves that ultimately killed the fish and terminated the trials. Though mosquitofish are generally considered hardy, temperatures outside of their optimal range can have detrimental effects on fitness and can be fatal (Cech et al., 1985; Vondracek et al., 1988). Climate change is affecting the broader agricultural landscape, and extreme temperatures and unpredictable weather are becoming factors that growers are already having to contend with in established agricultural practices (Jackson et al., 2011; Pathak et al., 2018). Rice fields have, historically, been flooded later in drought years (Reiter et al., 2018). Delayed floods increase the risk of encountering higher temperatures in the early season. If warmer temperatures increase the rate of development in TPS, as is often the case with arthropods, increased rates of TPS would shorten the release window even further since the shrimp could grow to injurious sizes quicker. Even if the fish are released within an actionable window, the TPS may also quickly outgrow the mosquitofish's ability to eat them.

Separate from the logistics, mosquitofish may also simply be a poorly matched choice for TPS control. Though they are heavily used for vector control, mosquitofish are generalist

predators that will consume many types of prey, potentially reducing their impact on a given species or pest (Kumar et al., 2015; Sutton et al., n.d.). In California rice fields specifically, feeding on mosquito larvae diminished in the presence of zooplankton (Bence, 1988). It is very possible that the abundance and diversity of aquatic arthropods in rice fields would diminish their TPS predation (Kumar et al., 2015; Linden and Cech, 1990). Furthermore, though our feeding assays indicated reasonable hunting success, our assay arenas were designed to exclude as many wild, alternative prey items as possible. Similarly, though Bloese et al. achieved control of TPS with mosquitofish, their field trials used arenas (1m²) much smaller than our field plots (100ft² or 9.24m²). A reduced arena size is likely easier for visual hunters like the mosquitofish as there is less overall space to search or for prey to hide. Additionally, there are likely fewer available alternative prey items to distract the fish. That, coupled with fewer shrimp to begin with, could have contributed to high success rates in previous studies, or detracted from the potential success of our own.

Furthermore, biological control of aquatic invertebrates is rare, but aquatic biological control efforts broadly speaking have a higher use of native vs. non-native control agents (Bajer, 2019). However, TPS are crustaceans evolved to live in vernal/ephemeral water sources with few to no natural enemies, particularly no known fish species (Boix et al., 2006). Finding aquatic natural enemies to serve as biocontrol for TPS would be difficult because they would also have to be adapted to the same ephemeral environment. There are some predacious arthropods, as mentioned, which are found at some abundance in rice fields that may be a better option for a biological control agent. Copepods are currently being considered as biological control agents for mosquito larvae (Pauly et al., 2022). If copepods are present in fields at flooding, it is possible that certain species may be able to provide some TPS control while they are in the

neonate phase. *Daphnia magna* do have morphological responses to a related tadpole shrimp species (*Triops cancriformis*) to defend themselves from predation by mature TPS (Rabus & Laforsch, 2011). If copepods are to be investigated further, sufficient research into species and their interactions with TPS must be considered. California rice fields double as wetland habitat for many migratory bird species which could serve as potential candidate for a biological control agent. In Spain, roseate spoonbills have been observed feeding on small crustaceans, including local tadpole shrimp species, in wetland habitats (Surdo, 2020). The direct manipulation or release of a vertebrate species is more challenging than with invertebrates, so birds may be more useful through conservation control methods rather than traditional release methods. However, again, careful consideration would be required to ensure that bird feeding habits are not destructive towards the rice seedlings as well.

Augmentative control has had many documented successes in a variety of agricultural settings, but California rice as a crop system may not be ideal for this method of control. Augmentative biocontrol tends to work well in closed systems, such as greenhouses, in which natural enemy populations cannot disperse, pest populations are functionally limited, and alternative prey options are scarce (Michaud, 2018; Van Lenteren, 2012b). The rice system, despite being an open agricultural field, almost behaves as a closed system. TPS won't migrate in from outside of field and, similarly, TPS and released predators are more or less confined to a single field. On the other hand, rice fields can be quite large, requiring either high-density predator releases or an especially efficient predator with a high consumption rate that disperses well. Augmentation success has also been shown to be affected by the landscape context, but the extent to which landscape is a factor is dependent on the system itself (Perez-Alvarez et al., 2019; Costamagna et al., 2015). In cabbage, complex landscapes supported the released

predators with naturally occurring populations of other predatory species that increased suppression of the pest complex. Cabbage is an open system, like rice, and benefits from a combination of both augmentation and conservation biocontrol (Perez-Alvarez et al., 2019). The melon aphid study found a positive correlation between predator abundance and the presence of alfalfa in the surrounding landscape, as well as a higher abundance of aphids associated with non-crop habitat (Costamagna et al., 2015). If landscape complexity supports augmentative releases by supplementing natural enemy populations, rice being an aquatic crop is likely limiting any benefits supplied from the surrounding landscape. This is another way in which rice is almost acting as a closed system. The rice cultivation zone in California is not very diverse to begin with, as the clay soil is not well suited for other crops (Pittelkow et al., 2012). The surrounding agricultural land is also not likely to support natural enemies or predators of the TPS given that it is aquatic. Rice fields would also be nearly inaccessible to any predatory species supported by non-crop wetland habitat in the area. Ultimately, augmentative control works best either in a closed system, or when supported by naturally occurring predator species in a complex, open landscape. Despite having characteristics of both systems, rice receives little to none of the benefits of either.

Like any other annual crop, predator releases in rice must be repeated on a yearly basis. In other annual systems, growers may be able to use insectary plantings or otherwise maintain a diverse landscape that can help keep natural enemies in the area, then do inundative releases at the start of the season to help bolster those populations and achieve control (Wiedenmann & Smith, 1997). In rice, this would be a much harder task to achieve given that the pest is purely aquatic. If growers are able to use a fish species as a biocontrol agent, they may have to depend almost entirely on inundative releases, as keeping a sufficient enough population in the irrigation

canals may not be possible from year to year. However, these populations are not always reliable on a yearly basis and growers would still have to rely on releases. Developing mass rearing techniques for these species would be an additional challenge to overcome. Moreover, their predatory, aquatic life stage would have to align with TPS presence in the early season. Using aquatic arthropods does not necessarily circumvent the issue of a short release window.

Mosquitofish did not demonstrate potential as biological control agents in these trials. Further exploration into *Gambusia affinis* is likely not worthwhile, but other fish or arthropod species may be better suited for control of this pest. Augmentative biocontrol, however, does not seem to be particularly well suited to this system. Challenges encountered in these trials with mosquitofish will likely occur with other predator species. That being said, non-chemical control measures are still needed in this system. Organic producers would especially benefit from the development of biological control practices, as they are all but excluded in the current IPM framework. Further development of scouting practices to better identify infestation rates in the field are also needed as successful management plans hinge on the accurate assessment of the situation. The rice cropping system could also benefit from additional agroecosystem studies to better understand what factors could help support populations of other potential natural enemies.

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Tables:

Table 10: Trial naming conventions and experiment description

Trial Name	Experiment Type	Year	Field
Trial A	Density	2022	J7
Trial B	Density	2022	Systems
Trial C	Density	2023	Systems
Trial D	Timing	2023	J7

Figures:



Figure 25: Photo of field layout of fish density experiments. Stars indicate sampling location within each ring. Trials A and B sampled bottom left and top right corners only. Trials C and D sampled all four corners.

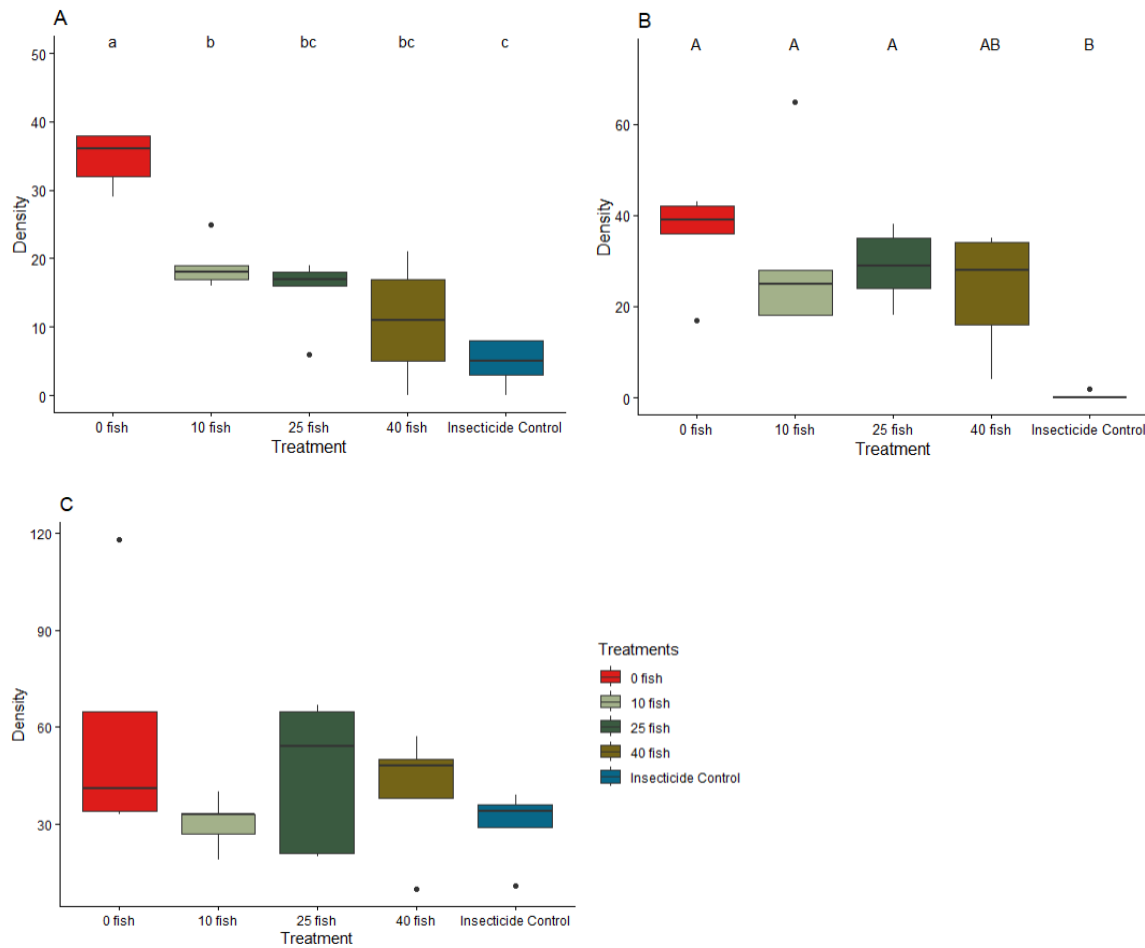


Figure 26: Overall density of tadpole shrimp by treatment for each fish density trial. Daily TPS densities were summed across reps for each day of the trials. Individual plots are labeled A, B, and C for their associated trials. Significant differences among treatments were identified with Tukey's post-hoc analysis ($\alpha = 0.05$). Letters indicate significance. In Trial A, the insecticide control treatment achieved the highest level of control, with the 40 and 25 fish treatments performing similarly. In Trial B, the insecticide control treatment again achieved the highest level of control and the 40 fish density performed similarly. In Trial C, there were no significant differences between treatments.

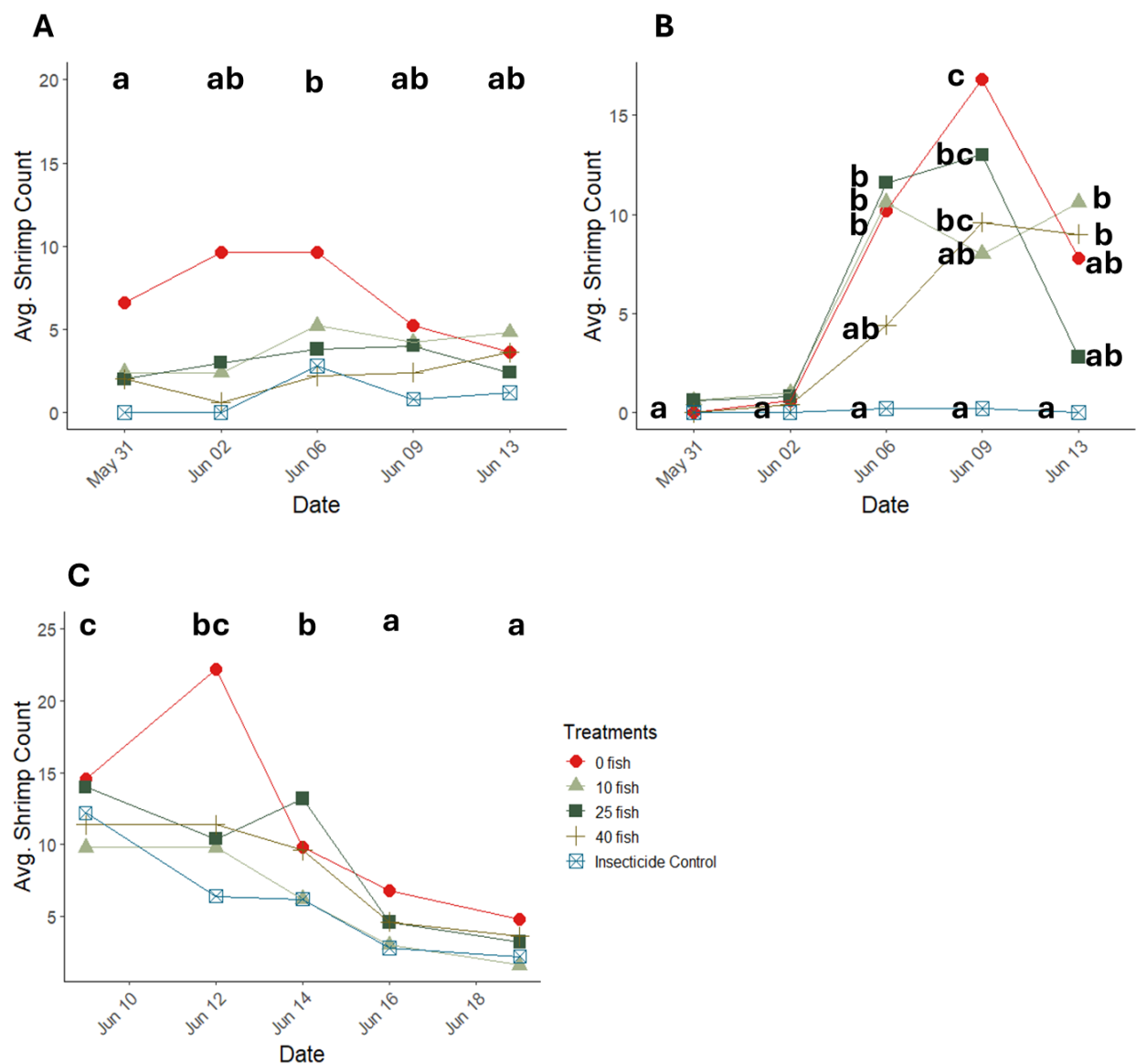


Figure 27: Average daily TPS capture by treatment. Individual plots are labeled A, B, and C for their associated trials. Significant differences among treatments were identified with Tukey's post-hoc analysis ($\alpha = 0.05$). Letters indicate significance. In Trial B, the interaction term was significant and significance codes are placed accordingly.

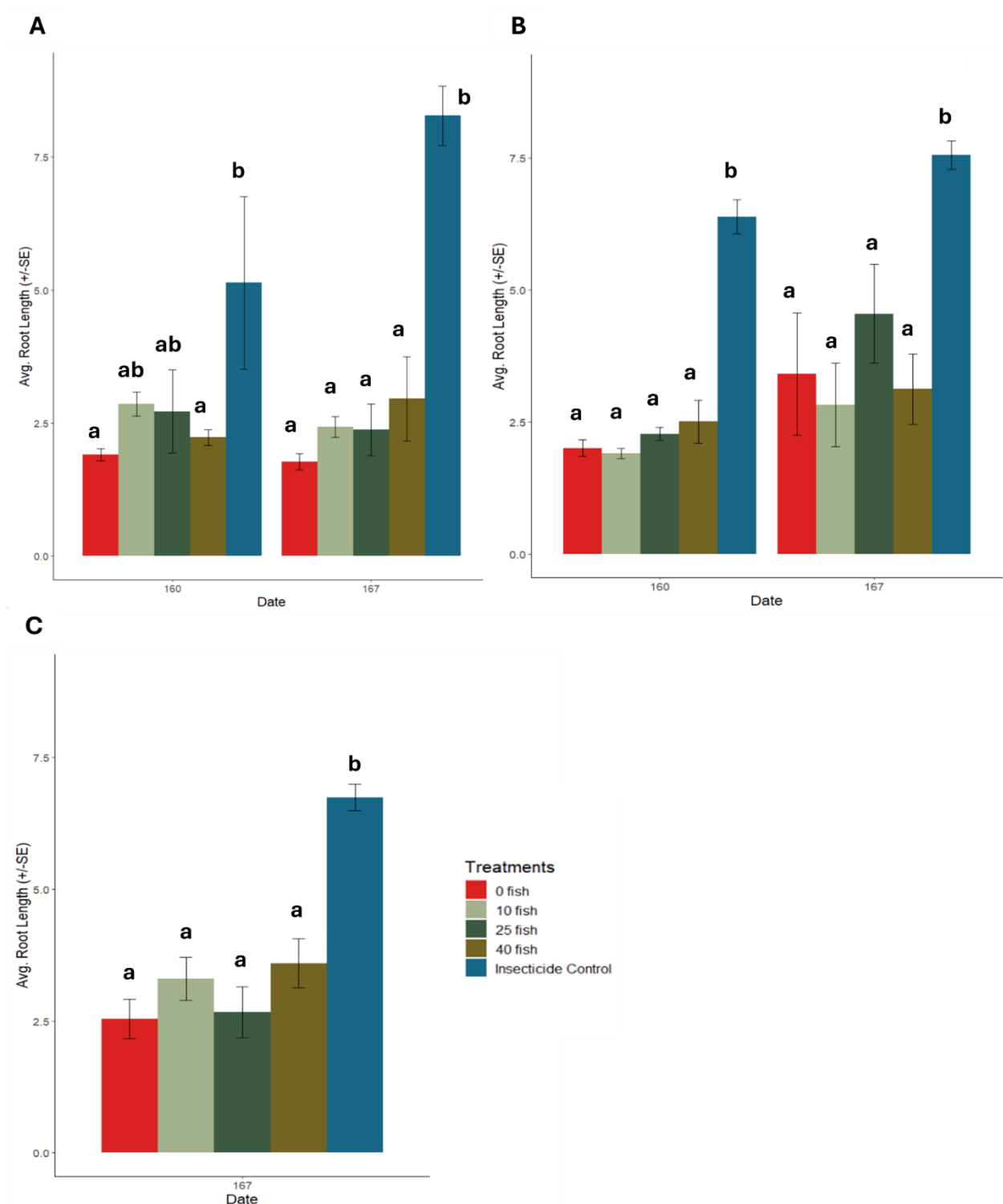


Figure 28: Average seedling root length with standard error in all 3 fish density trials. Individual plots are labeled A, B, and C for their associated trials. Significant differences among treatments were identified with Tukey's post-hoc analysis ($\alpha = 0.05$). Letters indicate significant difference in treatments. There was only one collection date in Trial C.

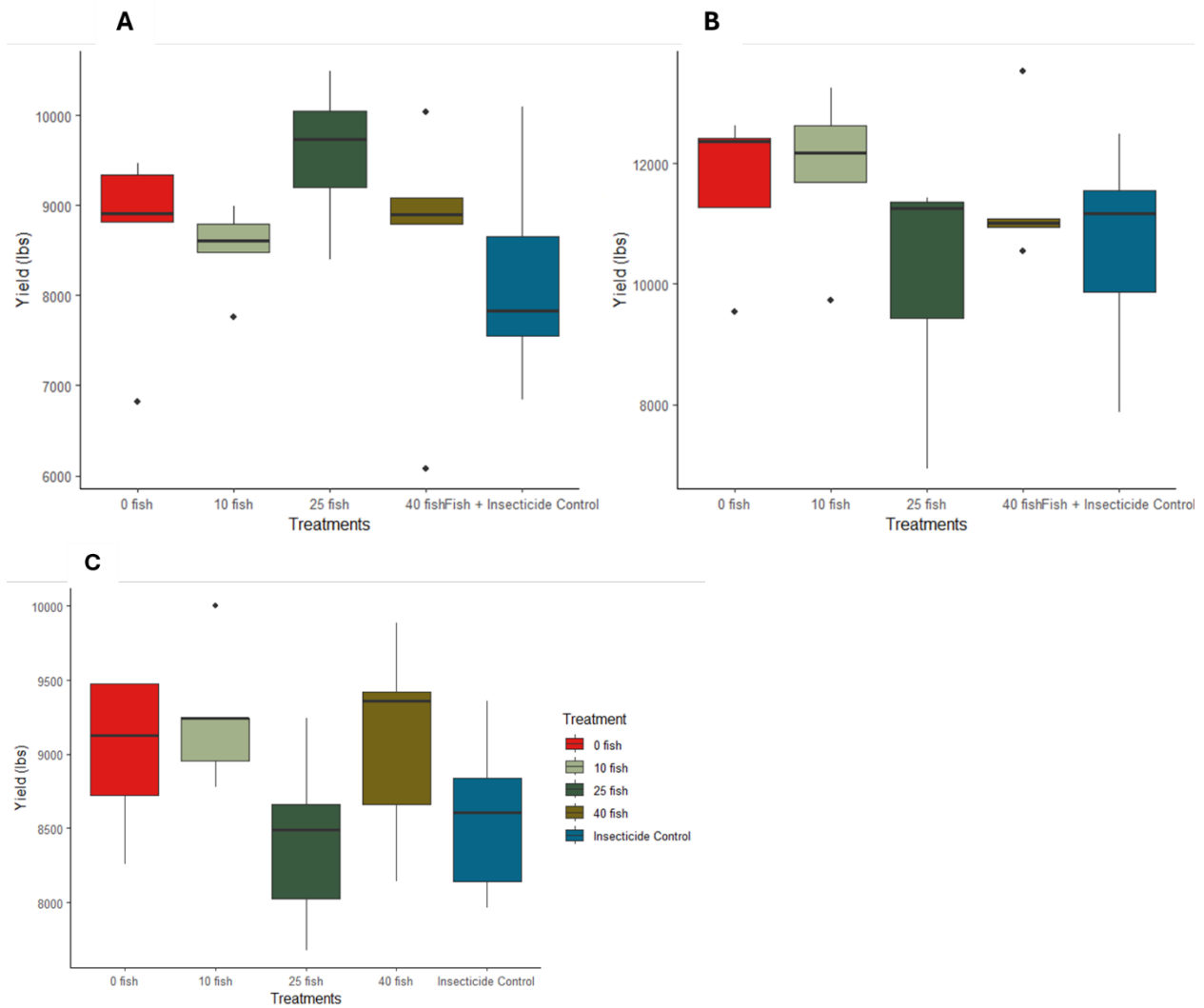


Figure 29: Rice yield by treatment for fish density trials. Individual plots are labeled A, B, and C for their associated trials. Treatments were tested with Tukey's post-hoc analysis ($\alpha = 0.05$). No significant differences were identified in any trial.

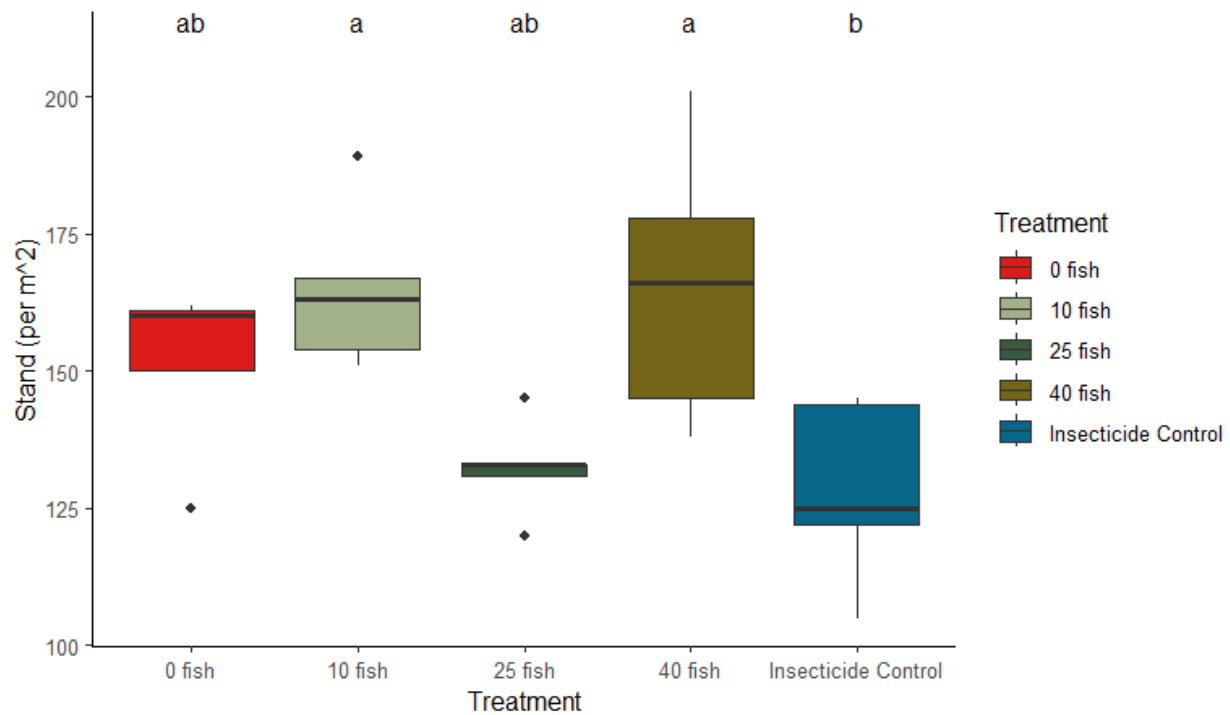


Figure 30: Rice stand count per m² from Trial C. Tukey's post-hoc analysis ($\alpha = 0.05$) was used to identify differences between treatments. Letters indicate significant differences in treatments.

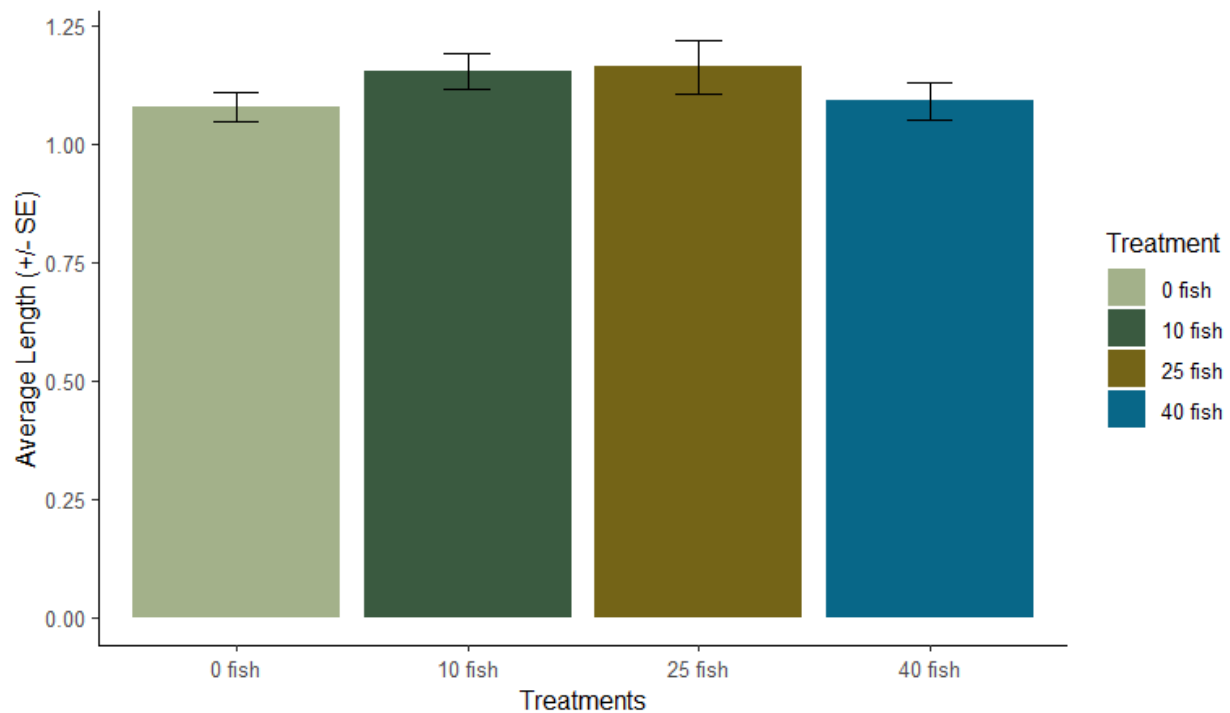


Figure 31: Average shrimp length in cm with standard error from Trial C. Shrimp were measured from tip of carapace to tail, excluding filaments. Tukey's post-hoc analysis was performed ($\alpha = 0.05$). There were no significant differences in shrimp length.

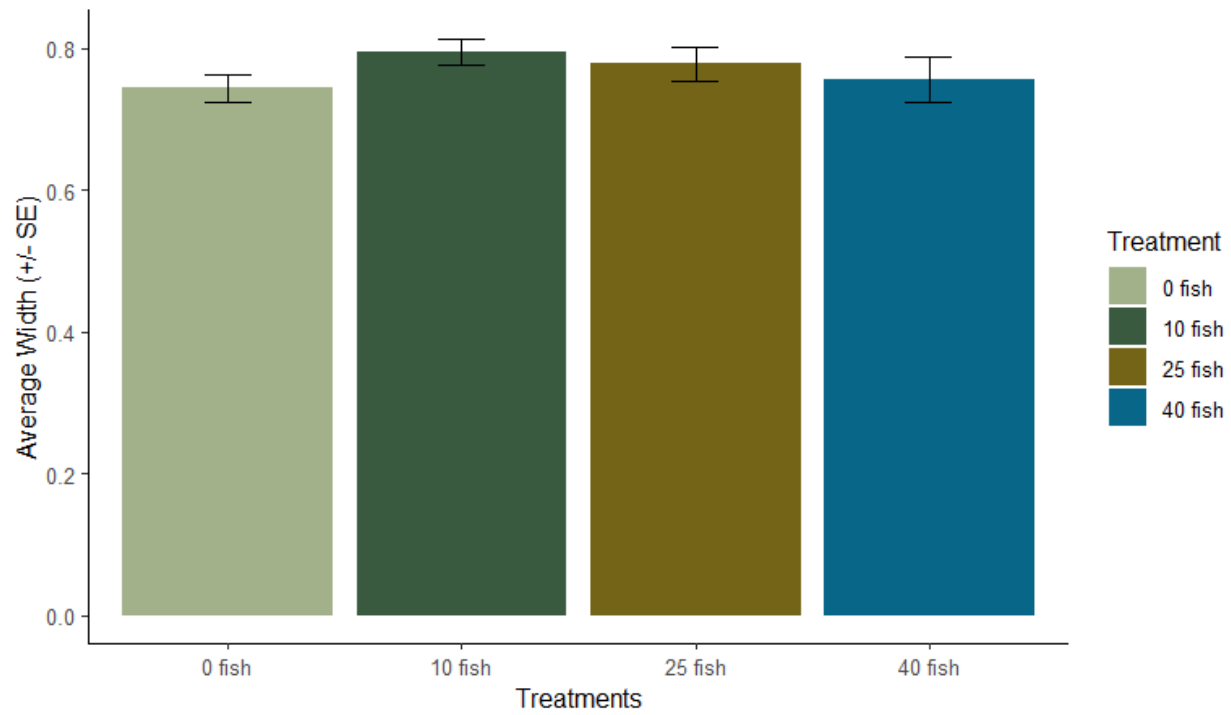


Figure 32: Average shrimp width in cm with standard error from Trial C. Width was measured across widest part of the carapace. Tukey's post-hoc analysis was performed ($\alpha = 0.05$). There were no significant differences in shrimp length.

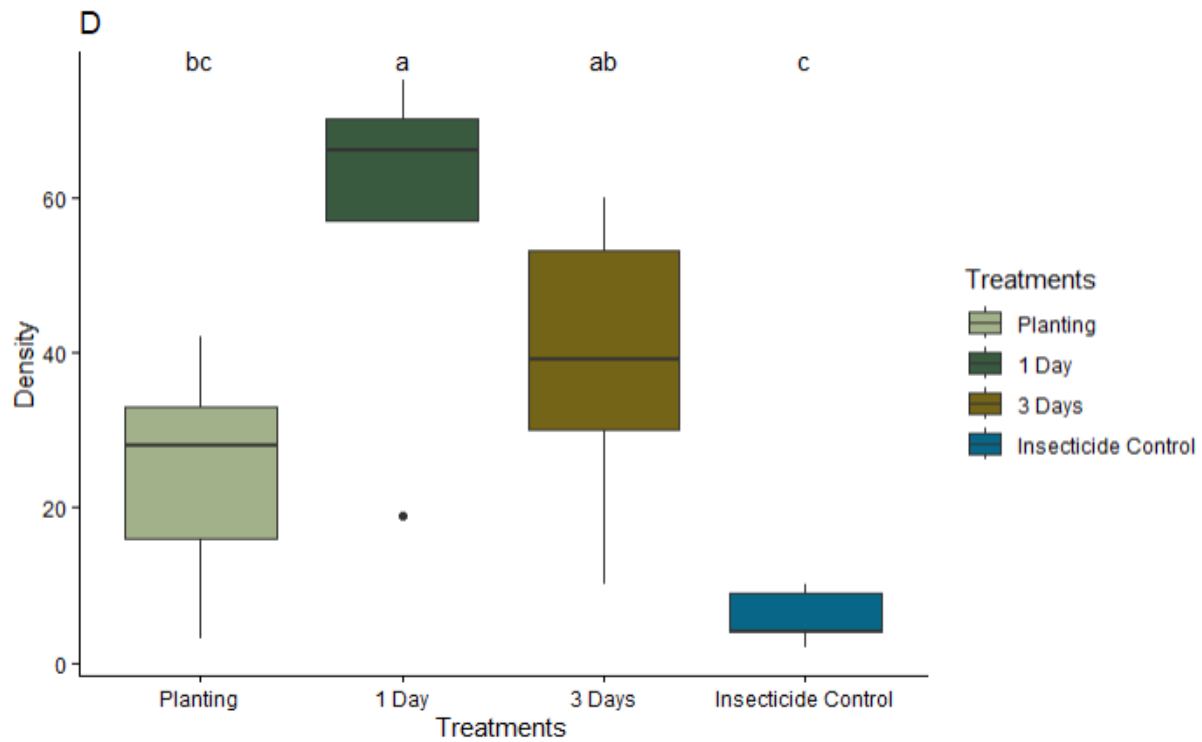


Figure 33: Overall density of tadpole shrimp by treatment in Trial D. Daily TPS densities were summed across reps for each day of the trials. Significant differences among treatments were identified with Tukey's post-hoc analysis ($\alpha = 0.05$). Letters indicate significance. The insecticide control treatment had the fewest number of TPS on average, with the Planting timing treatment performing similarly. The 1 Day After planting treatment performed the worst, likely due toxic herbicide drift, resulting in artificially higher numbers of shrimp in those treatments.

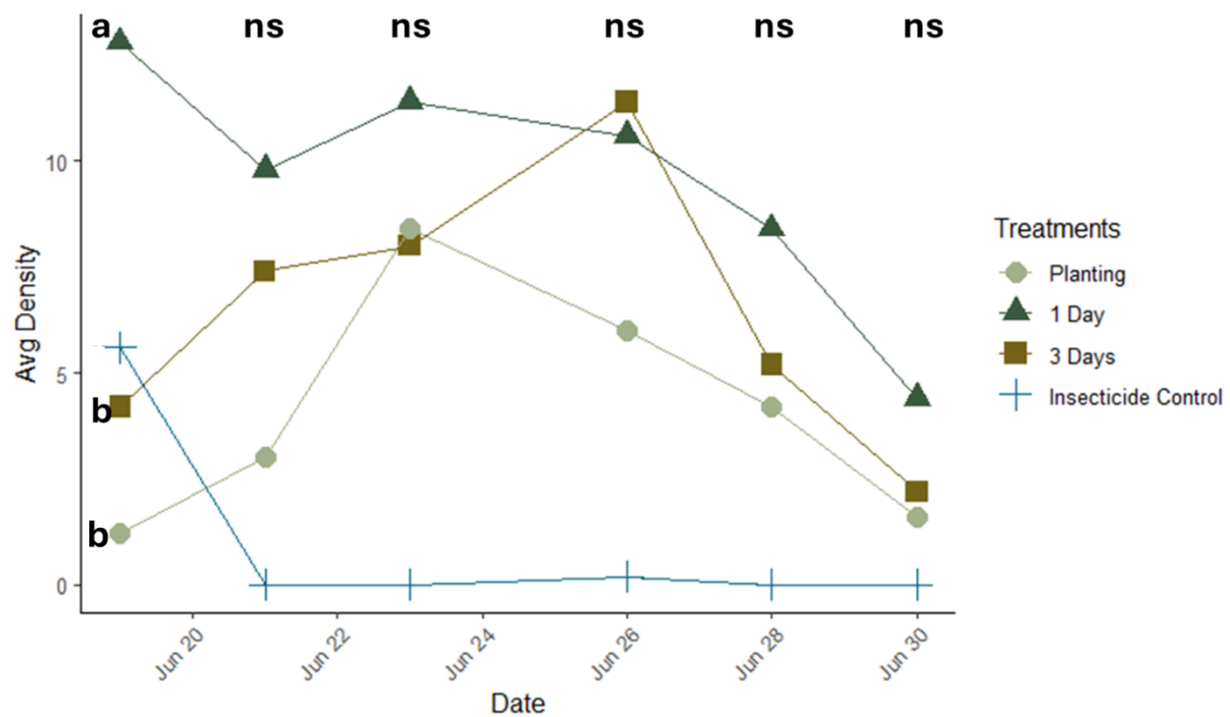


Figure 34: Average density of TPS by day in Trial D. Data was analyzed using mixed linear regression. Insecticide control treatment was removed from analysis. Significant differences among treatments were identified with Tukey's post-hoc analysis ($\alpha = 0.05$) Letters indicate significant differences between treatments on Day 1. The following days were not significantly different. The 1 Day After planting had more TPS to begin with than other treatments due to uneven exposure to toxic herbicide across the field.

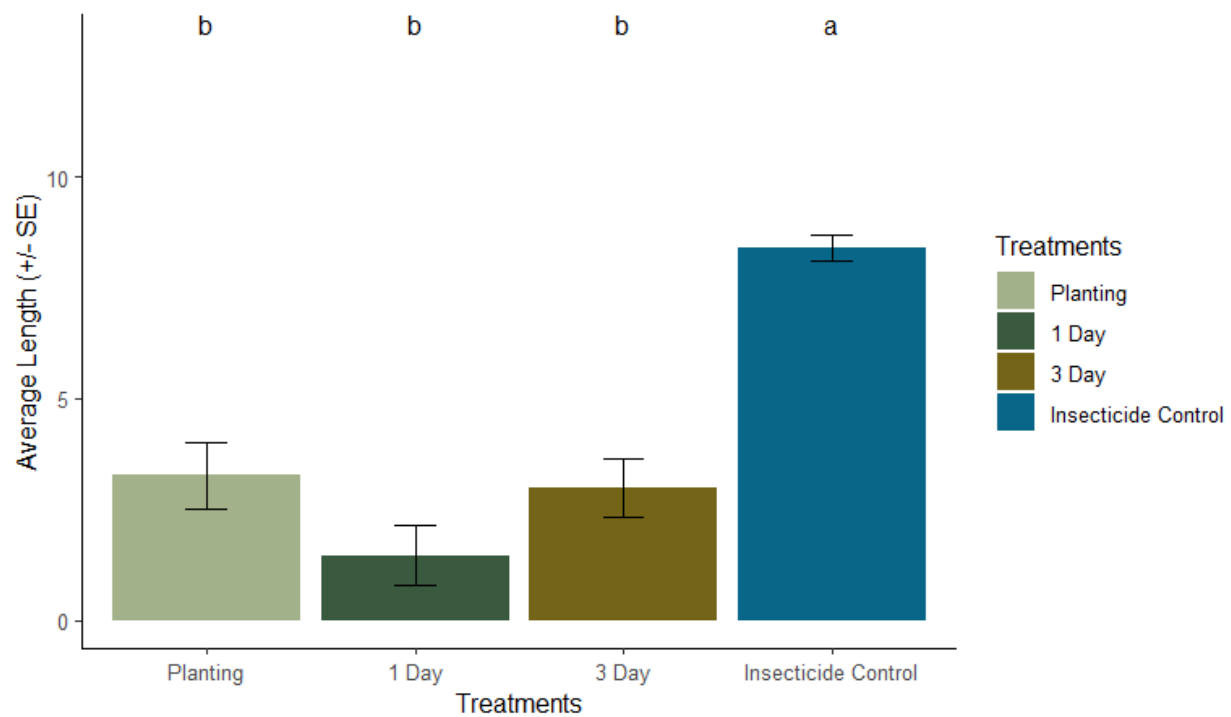


Figure 35: Trial D average seedling root length by treatment with standard error by treatment. Significant differences among treatments were identified with Tukey's post-hoc analysis ($\alpha = 0.05$) Letters indicate significant differences. Seedlings were, on average, longer in the insecticide control treatment. Other timing treatments incurred the same amount of damage and did not significantly differ.

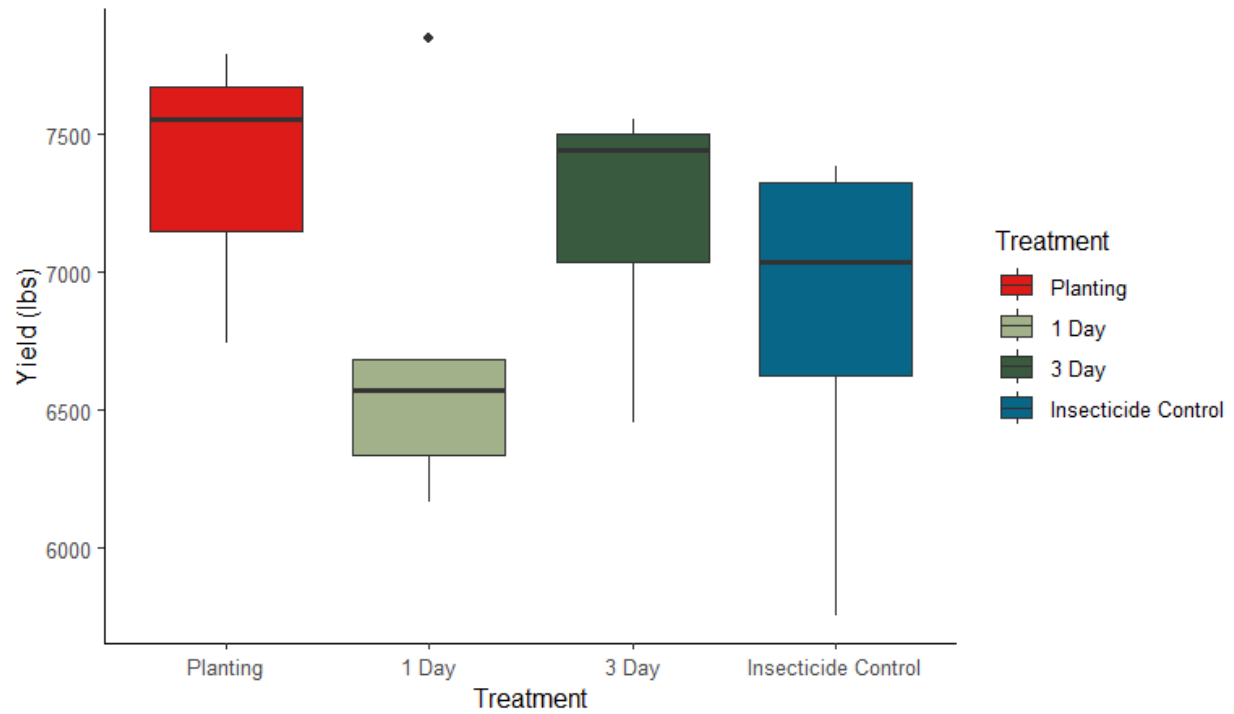


Figure 36: Trial D rice yield results in pounds. Data analyzed using a linear mixed effects model. Significant differences among treatments were identified with Tukey's post-hoc analysis ($\alpha = 0.05$). No significant difference between treatments.

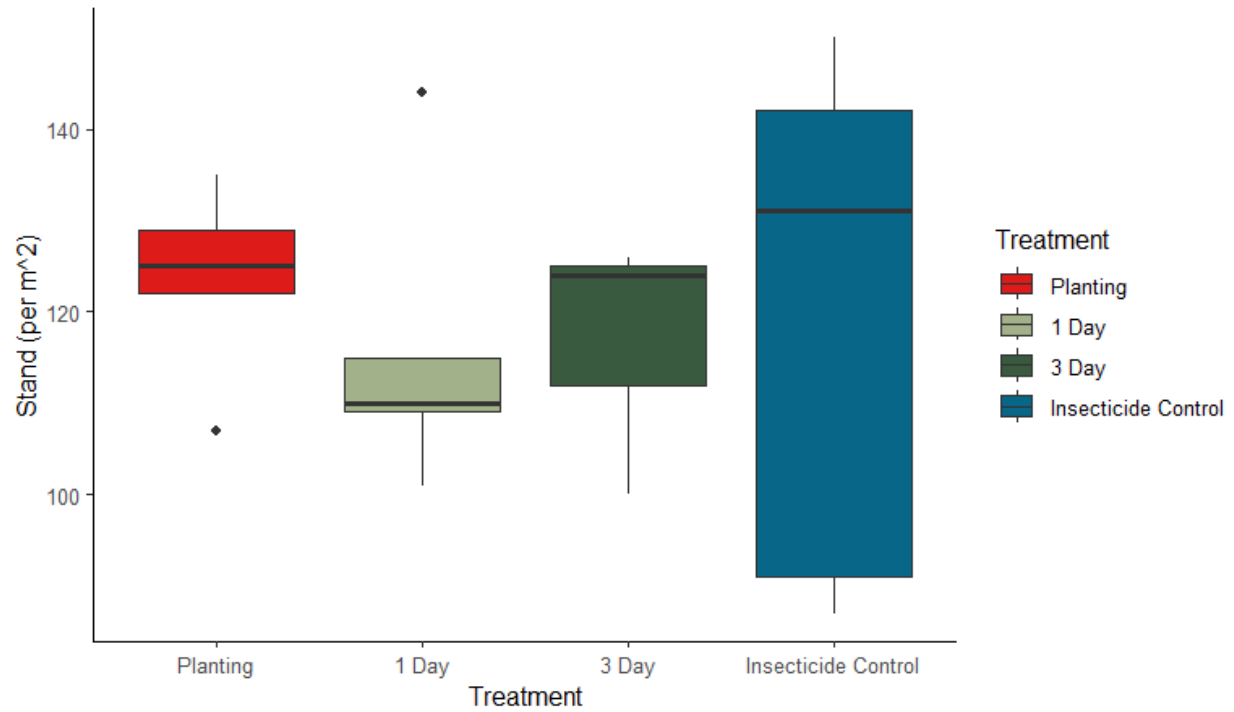


Figure 37: Trial D rice stand counts per m² results. Data analyzed using linear regression with Tukey's post-hoc analysis ($\alpha = 0.05$), but there were no significant differences between treatments.

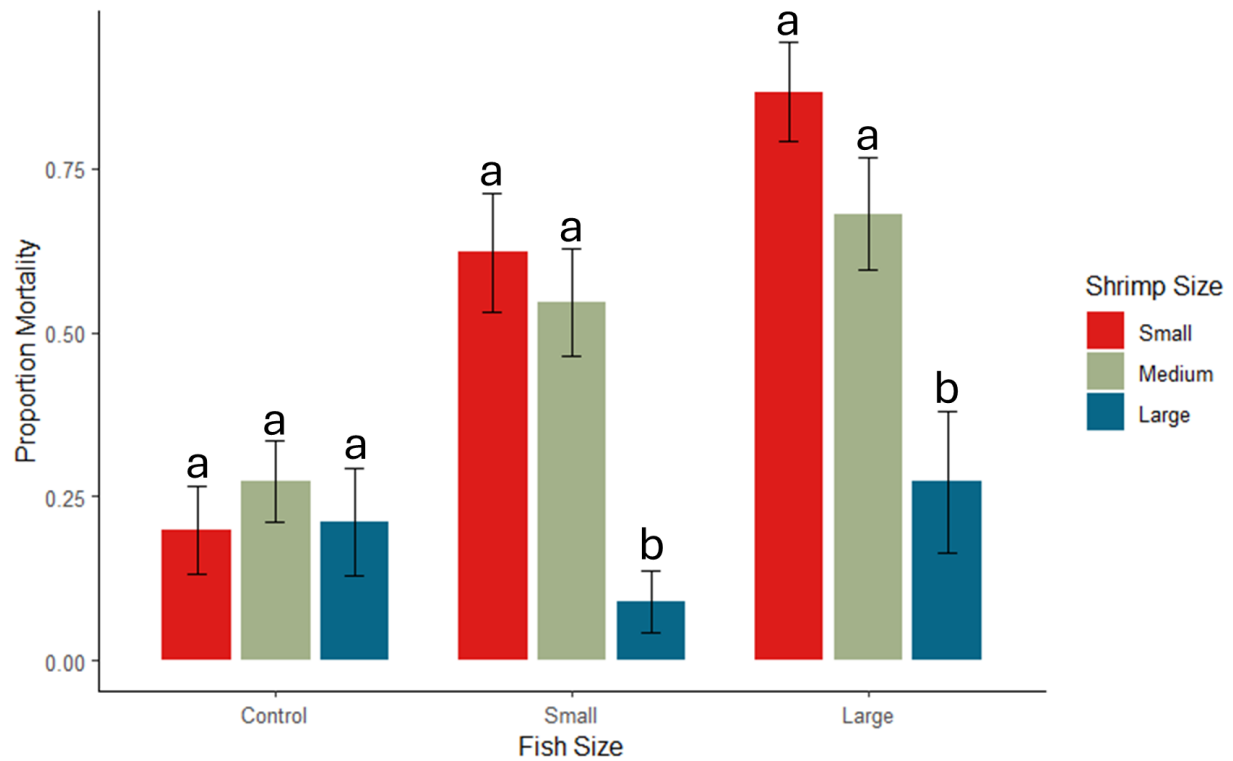


Figure 38: Fish feeding assay results with standard error from 2024 and 2025. Y axis represents the proportion of dead shrimp out of the total. Data analyzed using linear regression and pairwise comparisons performed using estimated marginal means ($\alpha = 0.05$). Letters indicate significant difference between treatments.