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# Determination of the relationship between the numbers of *Linepithema humile* (Hymenoptera: Formicidae) on irrigation pipes and tree trunks to facilitate automated monitoring in Southern California citrus orchards

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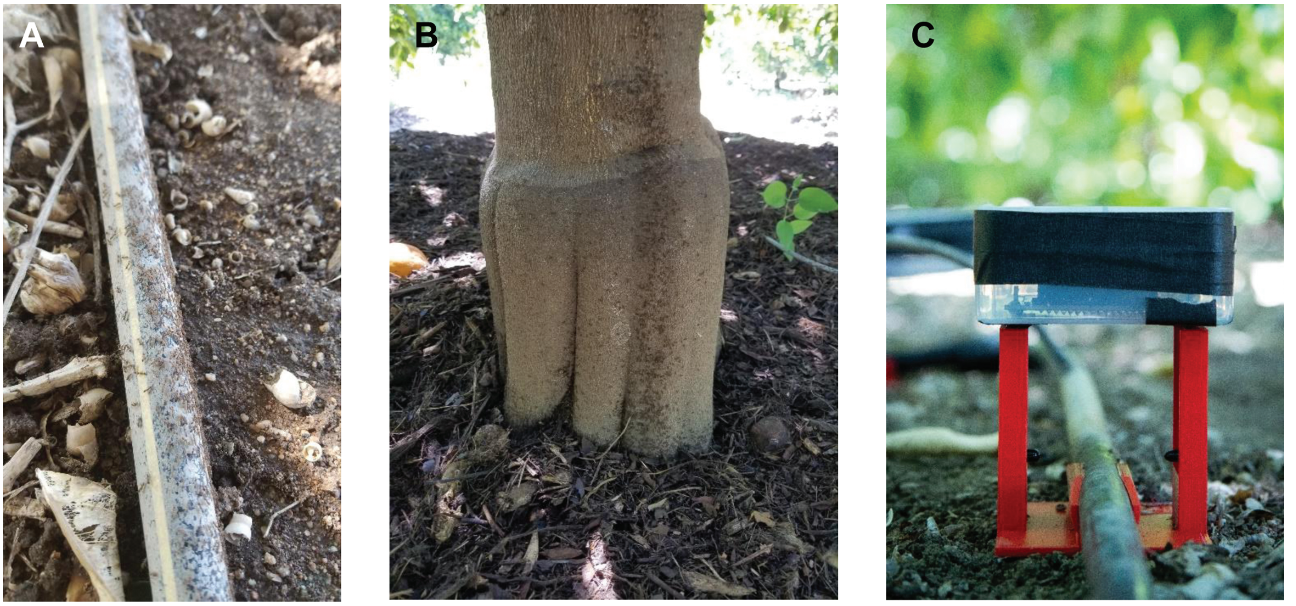
Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), is a pest in southern California citrus orchards because it protects honeydew-producing hemipteran pests from natural enemies. A major impediment to controlling *L. humile* is estimating ant densities in orchards. Ants use irrigation lines to travel across orchard floors to reach trees infested with hemipterans. However, for making ant control decisions, it is the number of ants in trees, not on pipes that is critical. Work completed here demonstrates that the number of ants counted on pipes is highly correlated with the number of ants counted on trunks. Densities of ants counted on trunks are correlated with trunk diameter, citrus variety, and time of year and time of day counts. Six regression models, linear regression, zero-inflated Poisson regression, and zero-inflated negative binomial regression models, and each of their mixed model extensions, indicated a strong positive relationship between ant counts on irrigation pipes and ant counts on tree trunks. Mean squared prediction error and 5-fold cross-validation analyses indicated that the best performing of these 6 models was the zero-inflated Poisson mixed regression model. A binary classification model developed using support vector machine learning for ant infestation severity levels, categorized as low (<100 ants counted in 1 min) or high (≥100 ants counted in minutes), predicted ant densities on trunks with 85% accuracy. These models can be used to estimate the number of ants on the trunks of citrus trees by using counts of ants made on irrigation pipes.

*Key words:* binary classification, cross-validation, machine learning, mean squared prediction error, regression analyses

## Graphical Abstract




(A) Argentine ants using a polyethylene irrigation pipe as a 'highway' to move across the floor of a commercial citrus orchard. (B) A trail of Argentine ants forming a column on the trunk of a citrus tree. (C) An infra-red sensor, attached to a polyethylene irrigation pipe, automates counts of ants on irrigation pipes.

Argentine ant, *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), native to subtropical South America, is a notoriously successful invasive tramp species that can thrive in wilderness, urban, and agricultural areas (Vega and Rust 2001, Silverman and Brightwell 2008). Once established, *L. humile* can cause significant ecological and economic damage in invaded regions, especially in areas with Mediterranean-like climates (Wetterer et al. 2009). *Linepithema humile* has been established widely around the world and within countries due to unintentional human-assisted movement (Ward et al. 2005, Wetterer et al. 2009). In agricultural zones, *L. humile* aggravates infestations of invasive phloem-feeding pest hemipterans through the formation of disruptive food for protection mutualisms. In this instance, *L. humile* protects sap-sucking pests from natural enemies, and in return, ants harvest honeydew, a liquid waste high in carbohydrates (i.e., soluble sugars), which foragers return to nests to feed nest mates (Moreno et al. 1987, Helms 2013, Tena et al. 2013, Yoo et al. 2013, McCalla et al. 2020). Because of this mutualistic relationship, the biological control potential of natural enemies is reduced by antagonistic interactions with ants (Milosavljević et al. 2021, McCalla et al. 2023). Consequently, hemipteran populations proliferate and produce more honeydew for ants to harvest, which in turn, promotes increased population growth of *L. humile*, which amplifies infestations and associated economic damage by both pest groups (Schall and Hoddle 2017, McCalla et al. 2023).

In California (USA), the citrus industry is estimated to have a total economic value of ~$7.1 billion per year (Babcock 2018). *Linepithema humile* has been present in California since at least 1905 (Smith 1936) and has developed mutualisms with several invasive and economically damaging honeydew producing hemipteran citrus pests (Markin 1970, Moreno et al. 1987, Yoo et al. 2013, McCalla et al. 2023). Control of *L. humile* in California citrus orchards relied primarily on a contact organophosphate insecticide, chlorpyrifos, which was banned in 2021 (Anon. 2020). The use of liquid bait stations to deliver very low concentrations of insecticide (e.g., boric acid, imidacloprid, methoprene, spinosad, or thiamethoxam) to foraging *L. humile* in a 25% sucrose water solution has demonstrated efficacy in citrus orchards with appreciable reductions in ant densities being achieved (i.e., >90% reduction in densities of foraging *L. humile*) (Klotz et al. 2000, Greenberg et al. 2013, Schall et al. 2018, McCalla 2019, McCalla et al. 2023). An alternative approach for delivering sucrose water laced with ultra-low concentrations of insecticide to *L. humile* in orchards is to infuse biodegradable alginate hydrogel beads (Tay et al. 2017, Schall et al. 2018, McCalla et al. 2020, Milosavljević et al. 2024) or polyacrylamide hydrogels (Buczkowski et al. 2014a, b, Rust et al. 2015, Boser et al. 2017, Tay et al. 2020) with toxicant. With both approaches, liquid bait stations and hydrogels, ants imbibe toxic sugar water and spread it through the colony via trophallaxis. When compared to untreated areas, treated areas result in rapid and sustained collapse of *L. humile* populations (McCalla et al. 2020, 2023, Milosavljević et al. 2024). Concomitant reductions in densities of hemipteran pests quickly result because of increased natural enemy activity (McCalla et al. 2023).

Despite *L. humile* being a well-recognized indirect pest in California citrus orchards, there are no standardized industry-adopted monitoring protocols to assess ant population densities and accordingly there are no action or economic thresholds to guide initiation of control treatments based on pest density estimates. Monitoring methods to estimate *L. humile* densities may utilize timed visual counts of ants passing a landmark on a tree trunk or use sugar water-filled monitoring vials to measure the number of ant visits to a sucrose resource over a 24-h period (McCalla et al. 2020). Both monitoring methods have shortcomings. Visual counts, especially when ant numbers are high, often lead to counting fatigue and inaccurate estimates. Moreover, they are neither cost- nor time-effective and only provide a temporal snapshot of ant activity based on the duration of the sampling interval (e.g., 1 min) and time of day (e.g., morning) counts are conducted (McCalla et al.

2020, Milosavljević et al. 2024). Subsequently, variation in diurnal and importantly nocturnal (Kistner et al. 2017) *L. humile* activity is not assessed. In comparison to visual counts, monitoring vials can measure changes in ant activity over time (e.g., 24 h). However, the deployment of vials of 25% sucrose solution recruits foragers to a highly preferred resource, which may artificially inflate estimates of ant densities while simultaneously redirecting ants away from study areas of interest (McCalla et al. 2020).

There is an obvious need for the development of new technologies to monitor *L. humile* activity in citrus orchards. Ideally, new approaches would be automated, able to accurately monitor hourly fluctuations in ant activity over a 24-h period, and ant count data would be transferable via cellular networks to cloud-based applications that analyze data and provide user-friendly summaries that are accessible via smart devices in near real-time. Additionally, field-deployed monitoring devices should have no effects on ant foraging behavior, be robust to environmental conditions (e.g., heat, irrigation water, and curious vertebrate animals), have low maintenance needs, require minimal power to operate, and componentry must be cheap, readily accessible, and easy to assemble.

Prototype infra-red sensors (i.e., IR sensors) currently undergoing field evaluation for *L. humile* monitoring in commercial California citrus orchards meet these criteria (Hoddle et al. 2022a). Strategic placement of IR sensors is critical, and highly rigid stereotypical foraging behavior of *L. humile* can be exploited for automating counts. Long runs of polyethylene irrigation pipes that lie in straight lines on top of the soil under citrus trees are used as "super-highways" by foraging *L. humile* to move between nests and trees with aggregations of honeydew-producing hemipterans. The use of irrigation pipes by *L. humile* for travel reliably concentrates workers in a relatively small area of the orchard floor, and the use of pipes optimizes foraging routes by maximizing transit linearity and smooth surfaces facilitate speed of movement, which collectively minimize travel times between nests and food sources (Yates and Nonacs 2016, Clifton et al. 2020). Foraging ants exit pipes and walk relatively short distances over the soil to access tree trunks to ascend into canopies where honeydew is collected and moved to nests via a return trip on irrigation pipes. In contrast to pipes where ant activity is predictably and reliably concentrated, the number and position of forager trails on trunks varies over time, which makes automated monitoring of trunks challenging. However, it is ant activity in trees, not on pipes, that affects the efficacy of biological control services in citrus orchards.

To increase the accuracy of ant density estimates in trees based on ant activity on pipes, the relationship between ants moving on pipes and tree trunks in citrus orchards needs to be determined. The work presented here used field-collected data on ant activity on irrigation pipes and movement on tree trunks, which were statistically analyzed to determine if a relationship exists between these 2 parameters. The construction of a robust model that accurately describes the relationship between the number of ants counted on irrigation pipes and those found on tree trunks would enable the conversion of counts of ants on pipes (i.e., automated IR-sensor count data) to estimates of numbers of ants moving on tree trunks and ultimately foraging in citrus canopies where they harvest honeydew and disrupt biological control services provided by natural enemies. Describing and defining this relationship between the numbers of ants counted on irrigation pipes and observed on trunks is a critical step in developing new automated approaches to monitoring *L. humile* in citrus, and the results of work analyzing this pipe-trunk relationship are presented here.

## Materials and Methods

### Field Sites and Data Collection

Counts of *L. humile* moving on the surface of black 12.7-mm diameter polyethylene irrigation pipes lying on the soil surface under citrus trees and numbers of ants ascending and descending tree trunks were made in 28 commercial citrus orchards in Riverside and San Bernardino Counties, California, over the period October 2019 to June 2020. Ant counts on pipes and trunks were made for 30 trees in each orchard (one orchard had 32 trees surveyed) for a total of 842 trees surveyed. Five trees in each of 6 rows were used, and each tree for which ant counts were made was separated by 2 trees, resulting in counts being made for every third tree in a row. Trees for which counts were made were in parallel rows (i.e., tree rows were not separated by another tree row; they were immediately opposite to each other), and trees for which ant counts were made were aligned in columns across rows. Counts in blocks of trees were initiated at a minimum of 2 rows from the margin of the citrus block being assessed for ant activity.

A 2-person team made 1 min visual counts of *L. humile*. One person made visual counts on the irrigation pipe immediately opposite the trunk of one of the 30 trees in the block. The second person counted the number of ants that were moving in the trails past a landmark on the trunk of the selected tree that was closest to the irrigation pipe. These trails on trunks are referred to as columns. This trail or column of ants on the trunk closest to the irrigation pipe was designated as "Column 1," and this was the column formed by ants arriving and exiting onto the pipe from the tree of interest. Upon completion of these counts, both team members counted the number of ant columns on the trunk and timed 1 min counts of the number of ants in these additional columns ($n = 1$ to 11). In addition to ant counts, the following data were collected for each tree: (i) the date and time of day that counts commenced for each tree, (ii) the diameter (cm) of each trunk (larger diameter trunks have more space to accommodate a greater number of ant columns), (iii) the variety of citrus comprising the block that ant counts were made in (i.e., oranges, grapefruit, lemons, and mixed varietal blocks). These data for each variable category were used to develop or "train" statistical models assessing the relationships between the numbers of ants on pipes and on trunks.

A second data set, used to test or validate (see below) models developed from collected data as described above, was generated in an identical manner as the original data set. These validation data were collected from an additional 10 orchards in Riverside and San Bernardino Counties over the period April–May 2021 for an additional 300 surveyed trees (i.e., 30 trees per orchard were sampled in a manner identical to that as described above for the collection of the primary data set).

### Data Analyses: Visualization of Training Data

For the training data collected from Fall 2019 to Spring 2020, trees with missing field data (e.g., tree trunk diameter and ant counts in columns 2–11) were removed from analyses. This resulted in the use of data from 23 orchards for a total of 700 trees for counts of ants on pipes and on trunks (column 1 or all columns on a trunk combined). All statistical analyses presented here were done using R. 4.4.1, and a significance level of 0.05 was used in all statistical tests.

To visualize the data, a dodge histogram (i.e., a side-by-side bar chart) was generated and used for side-by-side comparisons across the distributions of ant counts from the pipe only, column 1 only, and all columns combined (Fig. 1). For model development, the "corrplot" package in R was used to generate a correlation plot

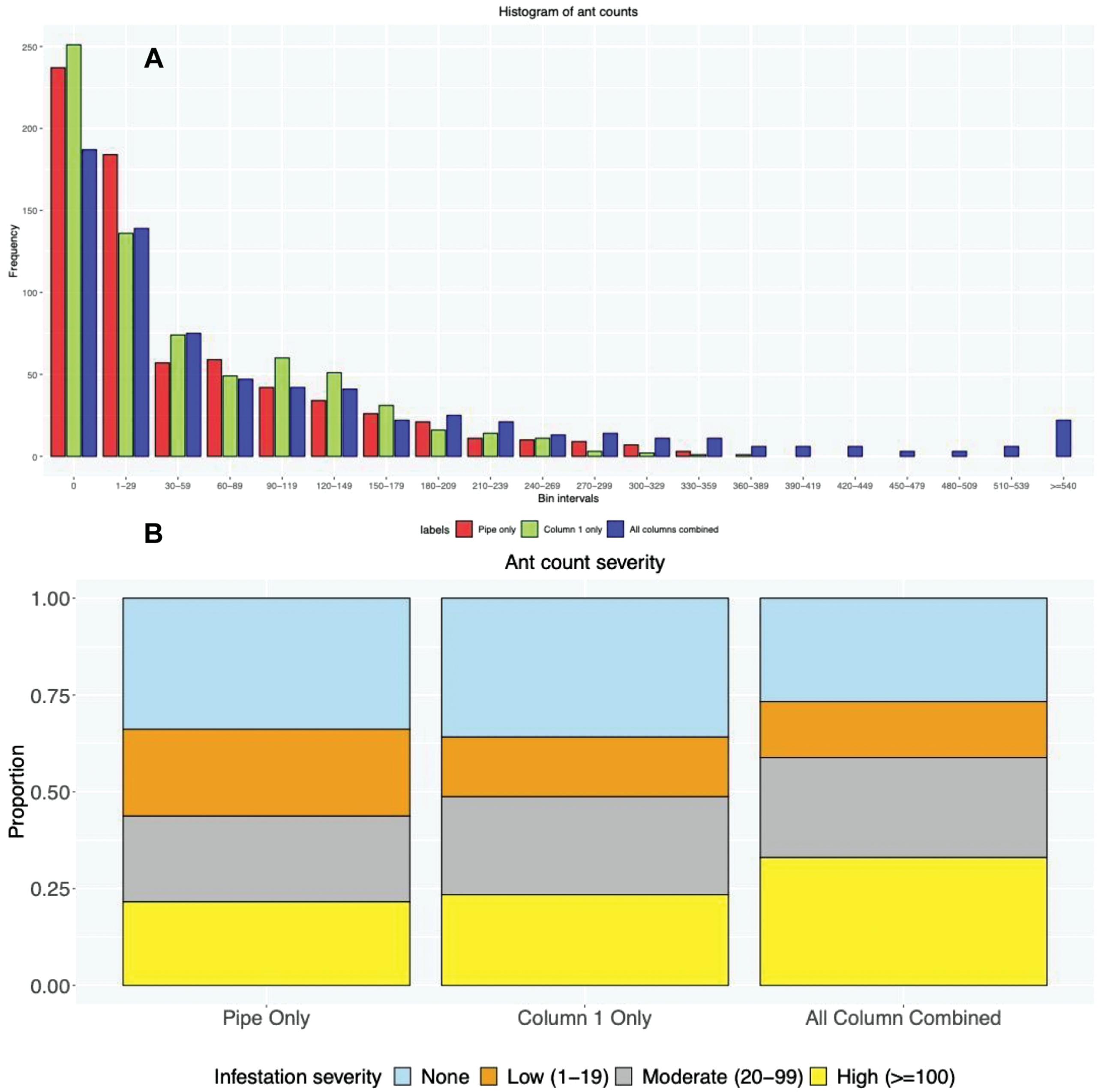



**Fig. 1.** A) Histogram of *Linepithema humile* counts that resulted from timed 1 min counts on irrigation pipes only, in column 1 on the tree trunk closest to the irrigation line, and for all ant columns combined. B) The proportion of *L. humile* in severity infestation category when counted for 1 min on irrigation pipes only, in column 1 on a tree trunk, and for all ant columns combined.

between ant counts on the pipe, ant counts in Column 1, ant counts from all columns combined, and the diameter of the tree. The correlation plot was used to determine if ant counts on the trunk (i.e., column 1 or all columns combined) were positively correlated with the total number of ants counted on the pipes, which was the focus of the modeling efforts detailed in the next section.

Because of potential measurement error due to the visual counting of ants, a categorical ant infestation severity index was generated, where the numbers of ants counted in 1 min on pipes, column 1 and all columns combined were categorized as none [i.e., zero ants counted], low [1–19 ants], moderate [20–99 ants], and high [≥100 ants]). The moderate (i.e., 20–99 ants) and high (i.e., ≥100 ants) thresholds were selected to create balanced cohorts of trees with respect to ant counts on trunks. Stacked barcharts using the "ggplot2" package of R were generated to compare distributions of 'infestation severity' between different seasons (i.e., fall [October 2019] vs. spring [April–June, 2020]), citrus types (i.e., oranges, grapefruit, lemons, or mixed unidentified varieties), and time of the day (i.e., morning vs. afternoon). These plots were generated for all ant counts on pipes in column 1 and all columns combined. Pearson's Chi-square tests (the "stat" package in R) were performed to determine if ant infestation severity was associated with season, time of day, and citrus variety.

## Data Analyses: Model Development

Model development was undertaken as a 2-part process. The first part was the determination of the relationship between the number of ants on tree trunks (ant counts in column 1 only, or all columns combined) and the number of ants counted on irrigation pipes while controlling for other confounding variables such as tree diameter, season, time of day, and citrus variety. These relationships were investigated by developing regression models that accommodated important features of the data, such as the count response variable, which, in some instances, had high zero counts for ants, and sampled trees in orchards, which had a shared orchard effect. Second, classification models were developed for infestation severity (i.e., none [zero ants counted], low [1–19 ants], moderate [20–99 ants], and high [≥100 ants]) by using ant count data on pipes so that model results which predict ant densities on trunks can provide estimates of ants moving on trunks into trees which can then be used for making control decisions. An additional benefit to using classification models is that accurate prediction of the ant counts on a highly infested tree using a regression model may not be necessary since ant counts [≥100 per minute] are subject to high levels of human counting error, ant densities ≥100 are likely problematic and increasing estimation accuracy (e.g., 180 ants vs. 250 ants per minute) will not change the conclusion that damaging ant densities have been observed.

### Development of Regression Models for Ant Count Data

Since the primary outcome of analyses is a count variable, 3 types of regression models, linear regression, Poisson regression, and negative binomial regression, were assessed for their abilities to model the relationship between ant counts on tree trunks and those on pipes while controlling for the effects of confounding variables (i.e., tree diameter, season, time of day, and citrus variety). The linear regression model was used as a baseline model against which outputs from the Poisson and negative binomial regression models were compared. Due to the excessive number of zero ant counts in column 1 and all ant columns combined on trunks, zero-inflated versions of the Poisson and negative binomial regression models were used for analyses, both of which were implemented using the "*zeroinfl*" function in the "*pscl*" package in R. Additionally, for each of the 3 basic types of models (i.e., linear, zero-inflated Poisson, and zero-inflated negative binomial), generalized linear mixed model extensions were also assessed (i.e., linear mixed, zero-inflated mixed Poisson, and zero-inflated mixed negative binomial). Mixed model analyses were conducted to account for intrinsic differences among sampled orchards by incorporating "orchard" as a random effect. This resulted in a total of 6 regression models being tested. The linear mixed model was fitted using the "*lmer*" function in the "*lme4*" package of R, while the zero-inflated Poisson mixed model and the zero-inflated negative binomial mixed model were fitted using the "*mixed_model*" function in the "*GLMMadaptive*" package of R.

For each of the 6 models, the following variables were considered as predictors: ant counts on the neighboring section of pipe in the same tree row where counts were also made, season, citrus variety, time of day counts were made, and the diameter of the trunk. Nearby counts of ants on adjacent sections of the monitored pipe were used to provide more information on ant presence and activity when the initial counts at the point of interest were zero. The reason for doing this was that ants may have been present but not at the time the visual count was done, and this "nearby" ant count approach used existing information on ant infestation levels on the pipe in the immediate vicinity of the point of interest. As such, a dummy variable "ant present on pipe" was included as a covariate in all models. The dummy variable was coded as 1 if an ant count on nearby pipe was nonzero and 0 if there were no ants present at the nearby ant count location. The performance of the 6 models was compared based on their mean squared prediction error (MSPE), which was calculated using 5-fold cross-validation. Specifically, data were randomly divided into 5 subgroups and used to train the models multiple times, with each training run using 4 subgroups (i.e., 80% of data) for training and the remaining subgroup (i.e., 20% of data) as validation data. The MSPE was then averaged over the 5 subgroups. To have every orchard represented equally in each subgroup, trees were randomized separately within each orchard when data splitting was performed. Finally, all candidate models were applied to the validation data collected in 2021 to assess robustness and generalizability.

### Use of Machine Learning for the Development of Classification Models for Infestation Severity

To develop classification models for the ant infestation severity levels (i.e., none [0 ants counted in 1 min], low [1–19], moderate [20–99], and high [≥100]), the out-of-sample classification performance of 2 most widely used machine learning methods, the multinomial logistic regression model and the multicategory support vector machine (SVM), were compared using both the training and test data sets.

Classification of ant infestation severity levels can be directly linked to ant management decision-making (i.e., treat or do not treat for ants). For example, a severe infestation level (i.e., ≥100 ants counted in 1 min) can result in the decision "treat," whereas a nonsevere infestation level (i.e., <100 ants) may result in a "do not treat" decision. Therefore, binary classifiers for nonsevere vs. severe infestation levels were constructed using both logistic regression and SVM.

## Results

### Visualization of Data

Distributions of the 3 ants count (pipes only, column one only, and all columns combined) were all right-skewed with ~67% of all observations being recorded in bin intervals <100 ants (Fig. 1A). The distribution plots of infestation severity (none, low, moderate, or high) for the 3 ant count variables (pipe only, column one only, and all columns combined) indicated a higher proportion of "high" infestations when all columns were combined (Fig. 1B).

The correlation between all numerical variables in the training data, including the 3 ant count variables and tree trunk diameter, was examined (Fig. 2). Trunk diameter had a mean width of 26.32 cm ± 8.71cm (SE) (min. = 4.0 cm, max. = 50 cm, median = 27.55 cm), and data had a nonnormal distribution (Shapiro–Walk's Normality test $P < 0.001$). The correlation plot suggested that there were strong correlations between ant counts on pipes and those on trunks (column 1 and all columns combined). This observation substantiated the underlying rationale of this study to build statistical and machine learning models predicting the ant counts on trunks using counts of ants on irrigation pipes. There were weak negative correlations between trunk diameter and ant counts, but these correlations were confounded by other factors, such as citrus variety. The importance of potential confounding effects was explored in subsequent regression analyses.

Stacked bar charts and chi-square tests were used to check if ant infestation severity (i.e., none, low, moderate, or high) was independent of other categorical variables such as season, citrus variety, and time of the day. Among the 700 trees retained in the training data set, 278 and 422 were surveyed in fall 2019 and spring 2020, respectively. Fig. 3A–C suggests there was a seasonal effect on ant infestation severity. A higher proportion of trees were classified as highly infested in spring when compared to fall. Chi-square tests confirmed that ant infestation severity levels were significantly affected

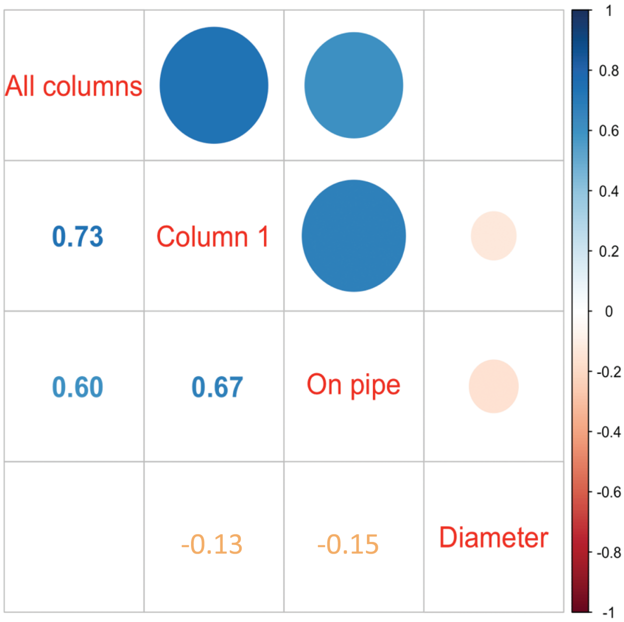



**Fig. 2.** Correlation plot between numerical variables in the training data, including ant counts on pipes, ant counts in column 1 only, ant counts in all columns combined, and the diameter of the tree trunks. The numbers in the plot are the correlation coefficients organized in the same order as in a correlation matrix. For example, −0.13 is the correlation coefficient between trunk diameter and column 1 ant counts.

by season for both ants in column 1 ($\chi^2 = 32.28$; $df = 3$; $P < 0.001$), and all columns combined ($\chi^2 = 33.66$; $df = 3$; $P < 0.001$). However, infestation ant severity on pipes was not significantly affected by season ($\chi^2 = 3.77$; $df = 3$; $P = 0.287$).

There were 371 orange trees, 212 grapefruit trees, 30 lemon trees, and 87 citrus trees with mixed unidentified varieties in the training data. Fig. 3D–F suggests that ant infestation severity differed across citrus varieties. There was a higher proportion of highly infested trees in blocks of oranges and mixed varieties. In contrast, there was a very small proportion of highly infested trees in lemons. Chi-square tests confirmed significant associations between citrus variety and ants on irrigation pipes ($\chi^2 = 72.23$; $df = 9$; $P < 0.001$), number of ants in column 1 ($\chi^2 = 63.34$; $df = 9$; $P < 0.001$), and total ants in all columns combined ($\chi^2 = 64.13$; $df = 9$; $P < 0.001$).

Trees and pipes were not assessed for ant activity at a consistent time period on each sampling event. A total of 415 trees were assessed for ant activity in the morning, and 285 were surveyed in the afternoon. Fig. 3G–I does not show an obvious difference between ant infestation severity levels in the morning when compared to the afternoon. However, chi-square test results showed a marginal significance for all columns combined ($\chi^2 = 8.68$; $df = 3$; $P = 0.034$) but not for ants on irrigation pipes ($\chi^2 = 4.05$; $df = 3$; $P = 0.256$) and ants in column 1 ($\chi^2 = 7.37$; $df = 3$; $P = 0.061$) with respect to time of day when counts were made.

## Regression Models

Regression analyses focused on the relationship between ant counts on tree trunks (column 1 only or all columns combined) and the number of ants counted on irrigation pipes. Results for regression and classification analyses for column 1 ant counts were similar to results for all columns combined. Consequently, results for column 1 ant counts are not considered further.

All 6 regression models, linear (mixed) regression, zero-inflated Poisson (mixed) regression, and zero-inflated negative binomial (mixed) regression models, indicated a strong positive relationship between ant counts on irrigation pipes and ant counts on tree trunks. To determine which model provided the best fit to the data, the MSPE for each of the 6 candidate models were compared.

For each candidate model, the MSPE for ant counts in all columns combined were compared using 5-fold cross-validation (Table 1). In addition to the overall MSPE, the MSPE for ant counts in each of the 4 ant infestation severity levels (i.e., none, low, moderate, and high) were also compared (Table 1). Based on the MSPEs, the zero-inflated Poisson mixed regression model, which took into account the random orchard effect, had the best fit to the training data (Table 1). The zero-inflated Poisson mixed regression model had the smallest overall prediction error, the smallest prediction error in 2 out of the 4 ant infestation severity levels (i.e., none and high) and the second-best performance in the remaining 2 levels (low and moderate ant severity levels).

The estimated coefficients of the zero-inflated Poisson mixed regression models using the full training data are presented in Table 2. The zero-inflated Poisson mixed regression model consists of 2 components: a binary logistic regression submodel (i.e., the zero-inflation model) to handle the excessive zero counts in the data and a Poisson mixed regression submodel with a log link function to predict nonzero ant counts. Both season (fall vs spring) and time of the day (morning vs afternoon) were binary predictors. The effects of the binary predictors reported in Table 2 represent the contrasts between spring versus fall and afternoon versus morning, respectively. Citrus variety was a categorical variable with 4 levels. Using grapefruit as the baseline, the effects associated with citrus variety in Table 2 were contrasts between orange versus grapefruit, lemon versus grapefruit, and mixed varieties versus grapefruit, respectively.

The results of these analyses suggest that the presence of ants on pipes and the actual counts on the pipe were both positively associated with the probability of a nonzero ant count on the trunk (Table 2). The likelihood of a nonzero ant count on the trunk in the spring and afternoon was greater when compared to ants being present in fall and morning, respectively (Table 2). There was also a higher likelihood of nonzero ant counts on the trunk of orange trees compared with other citrus varieties. Results of the Poisson mixed submodel also indicated that trunk diameter, ant counts on pipes, and the indicator of nonzero ant counts on pipes were all positively associated with nonzero ant counts on trunks.

To determine if these findings could be generalized, the 6 trained models, using the training data, were tested to predict ant counts from test data obtained in the Spring of 2021. Table 3 shows the MSPE of the 6 models used for testing the data, including the overall MSPE as well as the MSPE in 2 subgroups: (i) those with <100 ants counted per minute in all columns combined and (ii) those with ≥100 ants counted per minute in all columns combined. For all ant columns on trunks combined, the linear regression model had the best overall prediction performance, while the zero-inflated Poisson regression model was a close second in terms of performance (Table 3). The zero-inflated Poisson mixed regression model had the best prediction performance when ant counts on trunks were <100, an ant density category we are using here as being the most important for making control decisions (Table 3). The zero-inflated negative binomial regression model performed the best when ant counts on trunks were ≥100, a category that is subject to an increased likelihood of human counting error (Table 3). From a pest control perspective, accurate prediction of ant counts on trunks becomes less important when ant densities are

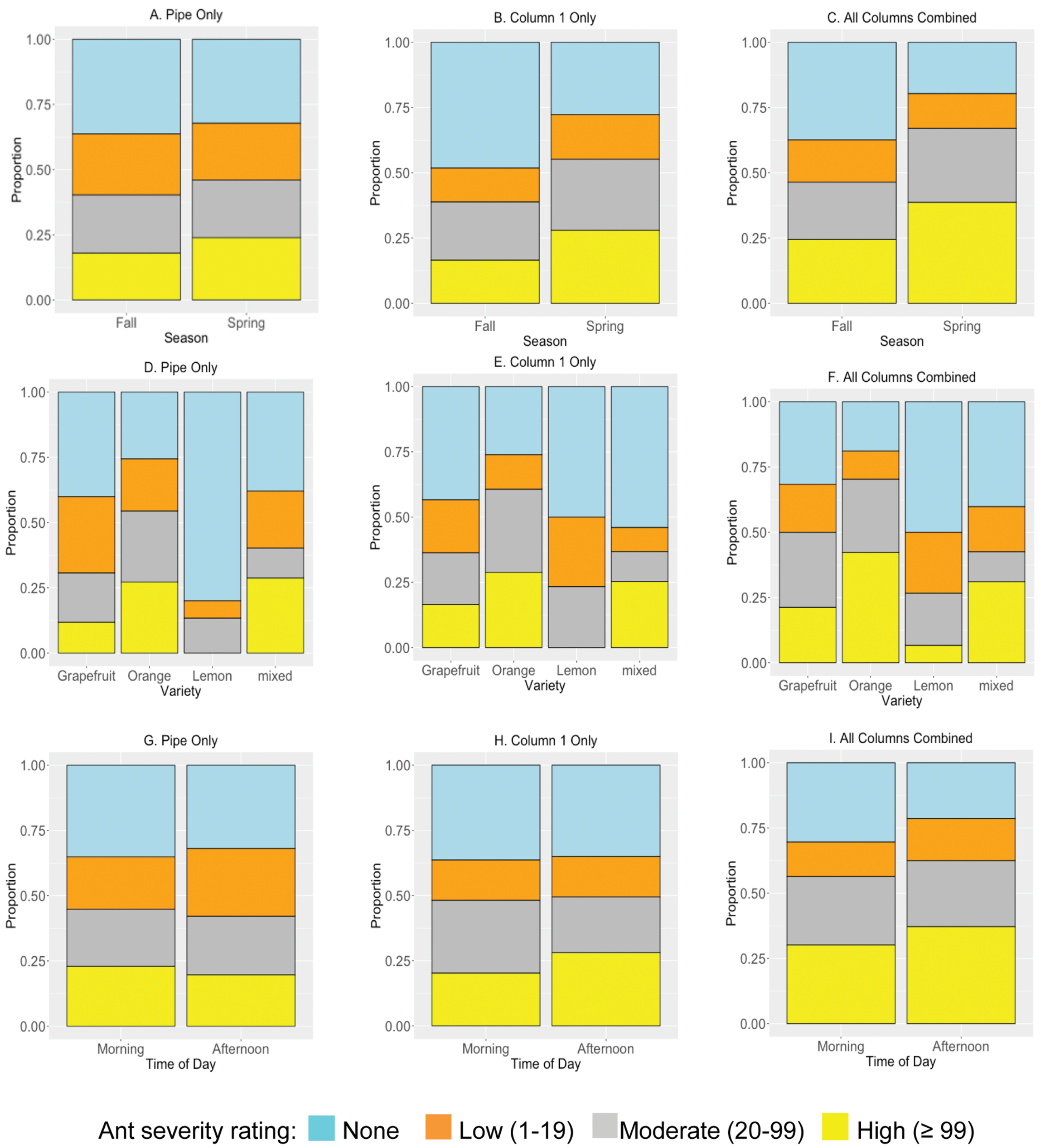



**Fig. 3.** *Linepithema humile* infestation severity graphs for season A–C), citrus variety D–F), and time of day ant counts were conducted G–I).

high (i.e., ≥100 ants counted per minute) because a less accurate prediction of the ant count will often lead to the same conclusion; ant densities are high and may need management. Therefore, these results from the test data support the conclusion that the zero-inflated Poisson mixed regression model provided the best overall fit to the ant count data.

## Classification Model Results from Machine Learning

The out-of-sample classification accuracy of the multinomial logistic regression model and the multicategory SVM were estimated to be 59.6% and 60.2%, respectively, for all ant columns combined for infestation severity categories (i.e., none, low, moderate, or high) based on 5-fold cross-validation using the training data. Additionally, both methods achieved 68.0% accuracy in the testing data collected in 2021. In comparison, a classifier can also be built using the predicted values of any of the regression models described in the previous section. The classifier based on the best regression model, the zero-inflated Poisson mixed model, had an accuracy of 47.6% based on a 5-fold cross-validation in the training data and 55.0% in the test data. In contrast to the regression models classifiers, the 2 machine learning classifiers had better performance as they were designed specifically for classification tasks.

**Table 1.** The MSPE comparison among 6 different regression models based on 5-fold cross-validation for numbers of ants counted visually over a 1-min period for all ant columns on trunks combined. Bold numbers are the smallest values in each row indicating the strongest model fits the data

| Ant infestation bin category (and severity rating) | Linear regression | Linear mixed regression | Zero-inflated Poisson regression | Zero-inflated mixed Poisson regression | Zero-inflated negative binomial regression | Zero-inflated mixed negative binomial regression |
|---|---|---|---|---|---|---|
| 0 | 2,772.86 | 1,456.69 | 2,112.18 | **650.81** | 1,933.89 | 958.90 |
| 1–19 (low) | 3,844.89 | 3,037.55 | 4,044.81 | 2,446.69 | 3,718.54 | **2,038.67** |
| 20–99 (moderate) | 8,574.06 | 6,734.37 | 5,384.08 | 4,299.43 | 8,618.51 | **2,678.52** |
| ≥100 (high) | 58,043.65 | 46,602.20 | 50,987.86 | **40,463.17** | 60,547.53 | 79,520.00 |
| Overall | 23,402.17 | 18,520.45 | 19,806.76 | **15,279.48** | 23,920.10 | 28,325.23 |

**Table 2.** Model estimations of the fitted zero-inflated Poisson mixed regression model, which is a mixture of 2 submodels—a logistic regression model (zero-inflation submodel) on the probability of a nonzero ant count on a tree trunk and a Poisson mixed regression submodel to predict the nonzero ant count on the tree trunk. The reported estimates are the regression coefficients of the predictors in the 2 submodels, respectively

| Predictors | Coefficients |
|---|---|
| **Poisson regression sub-model** | |
| Ants counted on pipes | 0.0045[a] |
| Trunk diameter | 0.0324[a] |
| Season: Spring | 0.9405 |
| Oranges | 0.5650 |
| Lemons | 0.0792 |
| Mixed citrus varieties | 0.5552 |
| Time of day | −0.0633[a] |
| Ants present on pipe | 0.0559[a] |
| **Zero-inflation sub-model** | |
| Ant counts on pipes | 0.0062[a] |
| Trunk diameter | 0.0112 |
| Season: Spring | 0.5153[a] |
| Oranges | 0.5586[a] |
| Lemons | 0.5550 |
| Mixed citrus varieties | −0.0494 |
| Time of day | 0.5444[a] |
| Ants present on pipe | 1.4751[a] |
| **Model evaluation** | |
| Amount of deviance explained | 65% |

[a] $P \leq 0.001$

**Table 3.** The MSPE for the 6 regression models was evaluated using test data obtained in Spring 2021. Bold numbers are the smallest values in each column indicating the strongest model fits the data

| Model | All ant columns on trunks combined | Ant counts on trunks < 100 | Ant counts on trunks ≥ 100 |
|---|---|---|---|
| Linear regression model | **6,546.62** | 2,912.58 | 14,268.95 |
| Linear mixed regression model | 8,719.14 | 4,194.98 | 18,332.97 |
| Zero-inflated Poisson regression model | 6,551.86 | 2,706.57 | 14,723.09 |
| Zero-inflated Poisson mixed regression model | 9,731.03 | **1,716.66** | 26,761.54 |
| Zero-inflated negative binomial regression model | 6,554.79 | 3,620.16 | **12,790.88** |
| Zero-inflated negative binomial mixed regression model | 7,506.78 | 1,838.04 | 19,552.85 |

Since the multinomial logistic regression model had comparable classification performance, and was more easily interpretable than the SVM, estimation results of the multinomial logistic regression using the full training data are presented in Table 4. The baseline infestation level used in the model was "none," and the parameters listed in Table 4 were the coefficients of various predictors in the log odds ratio between each ant infestation severity category (i.e., low, moderate, and high) versus "none." These results indicated that ants counted on pipes were highly informative for 2 reasons. First, knowing if there was a nonzero ant count on the irrigation pipe was highly predictive for the presence of ants on neighboring tree trunks. Second, visual ant counts on irrigation pipes were highly significant when classifying ant infestations as moderate or high on trunks. Additionally, both season and time of day were significant factors, and the results suggested that the infestation level tended to be higher in the afternoons in spring as opposed to mornings in fall, respectively. The diameter of the trunk was only significant for highly infested trees (≥100 ants counted in 1 min), and trees with larger trunk diameters had a greater tendency to be highly infested with ants. Finally, the results indicate that there is a greater likelihood of moderate to high infestation levels for orange trees when compared to other citrus varieties. There were no significant differences between grapefruit, lemon, and mixed citrus varieties. These results were consistent with findings from the regression analysis.

A contingency table between the true and predicted infestation levels based on the training data is presented in Table 5. Among the 4 ant infestation severity categories, the low infestation category (1–19 ants) was the most challenging for the logistic regression to correctly classify, as it only accounted for ~14% of all sampled trees in the training data. Of the total of 101 trees that had a low infestation severity rating (i.e., 1–19 ants counted per minute on trunks), the multinomial logistic regression misclassified 33, 38, and 8 trees to be in the infestation categories of none, moderate (20–99 ants) and high (>100 ants) infestation, respectively, and only 22 trees were correctly classified as having a low infestation of ants. The lower number of training samples in this relatively narrow interval likely resulted in the misclassification of these trees into neighboring, albeit incorrect classification categories. On the other hand, multinomial logistic regression did a good job classifying the highly infested trees. Out of the 231 trees that were highly infested, 170 trees (i.e., 73.6%) were correctly classified in this category.

To link classification results to pest control decisions (i.e., "treat" versus "do not treat"), a binary logistic regression and SVM were applied to classify ant counts as either nonsevere infestations (i.e., <100 ants counted per minute on trunks) versus severe infestation (i.e., ≥100 ants counted per minute on trunks). Based on 5-fold

**Table 4.** Estimated parameters from the multinomial logistic regression model where the dependent feature is all columns combined infestation severity (none [0 ants counted in 1 min], low [1–19 ants counted], moderate [20–99 ants counted], and high [≥100 ants counted])

| Predictors | Coefficients (low) | Coefficients (moderate) | Coefficients (high) |
|---|---|---|---|
| Ant counts on pipe | −0.0023 | 0.0222[c] | 0.0358[c] |
| Trunk diameter | 0.0147 | 0.0202 | 0.0516[b] |
| Season: spring | 0.9168[b] | 1.4518[c] | 1.9107[c] |
| Oranges | 0.4395 | 0.8546[a] | 1.8567[c] |
| Lemons | 0.7413 | 0.5725 | 1.0161 |
| Mixed citrus varieties | 0.1815 | −0.7204 | 0.2720 |
| Time of the day | 0.8444[a] | 1.0277[c] | 1.7829[c] |
| Ants present on pipes | 2.4015[c] | 2.7621[c] | 2.3699[c] |

[a]$P \le 0.05$;
[b]$P \le 0.01$;
[c]$P \le 0.001$.

**Table 5.** The contingency table of the predicted infestation levels using the multinomial logistic regression (rows) versus the true infestation levels (columns) for all columns combined for ant infestation severity in the training data

| | Ant infestation severity category | | | |
|---|---|---|---|---|
| Predicted severity category | None[a] | Low[a] | Moderate[a] | High[a] |
| None[a] | 151 | 33 | 24 | 11 |
| Low[a] | 11 | 22 | 8 | 1 |
| Moderate[a] | 20 | 38 | 92 | 49 |
| High[a] | 5 | 8 | 57 | 170 |
| Total number of trees | 187 | 101 | 181 | 231 |

[a]Where none = zero ants counted, low = 1–19 ants counted, moderate = 20–99 ants counted, and high was ≥100 ants counted visually in 1 min across all ant columns combined on citrus trunks.

cross-validation using the training data, the out-of-sample classification accuracy for ants counted in all columns on trunks combined and infestation severity was estimated to be 80.0% for the logistic regression model and 80.4% for SVM. The 2 binary classifiers achieved 84.7% and 87.4% accuracy for the infestation severity category for ants counted in all columns combined when using the test data collected in 2021. As the 2 binary classifier models had comparable classification, the estimated coefficients for the binary logistic regression model are presented in Table 6. These coefficients can be directly interpreted as log odds ratios. The conclusions drawn from the results presented in Table 6 were consistent with those obtained from the multinomial logistic regression analyses: visual timed 1 min ant counts on irrigation pipes, tree diameter, season (spring, fall), citrus variety (oranges, lemons, grapefruit, and mixed varieties) and time of the day (morning, afternoon) are all significantly associated with ant infestations categorized as "severe" (i.e., ≥100 ants counted per minute) for ants counts in all columns on trunks combined.

## Discussion

Results of regression analyses indicated strong statistical support for a robust association between the numbers of ants counted visually for 1 min on irrigation pipes and the numbers of ants counted

**Table 6.** Estimated coefficients of the logistic regression model for the binary classification of the total number of ants counted in all columns on trunks combined. The binary infestation severity categories used were nonsevere (0–99 ants counted per minute) vs. severe (≥100 ants counted per minute)

| Predictors | Coefficients |
|---|---|
| Ant counts on pipe | 0.0183[c] |
| Trunk diameter | 0.0303[a] |
| Season; spring | 0.7983[b] |
| Oranges | 1.2455[c] |
| Lemons | 0.4505 |
| Mixed citrus varieties | 0.5644 |
| Time of day | 0.9586[c] |
| Ants present on pipe | 0.7834[b] |

[a]$P \le 0.05$;
[b]$P \le 0.01$;
[c]$P \le 0.001$.

visually in all columns on tree trunks for 1 min in commercial citrus orchards in southern California. This relationship holds strongly when controlling for confounding factors such as the diameter of the trunk (i.e., tree size), season (spring and fall), time of the day (morning and afternoon), and citrus variety (oranges, lemons, grapefruit, and mixed varieties). Among the 6 regression models evaluated, the zero-inflated Poisson mixed model, which accounted for the orchard as a random effect, provided the best fit to data based on MSPE estimates.

To link the results of these regression analyses directly to ant management decision-making and to avoid overfitting models to high ant counts (i.e., ≥100 ants counted per minute), which are subject to a higher human counting error, classification models were used to assess ant infestation severity levels (none [0 ants counted in 1 min], low [1–19 ants], moderate [20–99 ants], or high [≥100 ants]). The multinomial logistic model, which had a classification accuracy of 68%, performed better than fitted regression models (i.e., 55% classification accuracy for the best regression model), provided additional insight into how different factors (e.g., citrus variety, trunk diameter) affected ant counts in the different ant infestation severity categories. However, ant counts on trees that fall into the low infestation bin category (i.e., 1–19 ants counted per minute) accounted for only 14% of the data, and this resulted in models misclassifying this category and placing low infestation measurements into neighboring bins (i.e., none or moderate severity categories). To circumvent this misclassification issue, especially at low ant densities where ant control decision-making is most critical, the binary logistic classifier was used.

The binary classifier directly predicts whether pest control action is needed based on whether ant counts fall into one of 2 infestation categories. The 2 infestation categories were classified as either nonsevere or severe. A nonsevere categorization, for example, would require no ant control treatments when <99 ants are counted in 1 min. Alternatively, ant control could be initiated, for example, when ant count densities are categorized as severe and ≥100 ants are counted per minute. The binary logistic classifier model achieved very high classification accuracy, 85%, in evaluation runs using field-collected testing data.

Our analyses focused initially on ant counts in column 1, the ant column on the trunk closest to the irrigation pipe ants used to move across the orchard floor. However, additional analyses indicated that total ant counts for all ant columns combined on trunks provided similar findings to analyses using count data for all ant columns combined. What is of most importance for ant management, and for analyses provided here, is the total number of ants counted in all columns (i.e., total ant load), not just the total number of ants in column 1, moving up trunks into citrus trees to tend hemipteran pests.

The practical applications of these findings can be used to estimate counts of ants on pipes made by IR sensors used to automate ant counts. The binary classifier nonsevere (i.e., <99 ants counted) vs. severe (≥100 ants counted per minute) predicted ant infestation severity level on trunks with 85% accuracy. These findings now enable statistical models to be programmed into software used to count and analyze IR sensor ant count data from irrigation pipes. These data, which can be uploaded and processed by cloud-based software, return density estimates of ants on pipes, which, using results from these analyses, can now be used to estimate the numbers of ants trunks of citrus trees. A binary classification output (85% accuracy), such as nonsevere (i.e., do not treat) vs. severe (i.e., treat), is possible. This simple and easy-to-understand output can assist with making decisions on whether or not management of Argentine ant is needed in commercial citrus orchards.

Additional research is needed if automated ant counts using IR sensors are to be realized. At this time, IR sensors have demonstrated proof of principle; they are able to make hourly ant counts and use orchard-based gateways and local cellular networks to transmit daily count data from citrus orchards to the cloud, where data can be accessed via an app. Cloud-based data for ant counts on irrigation pipes can be visualized and subsequently downloaded and analyzed (Hoddle et al. 2022a, b). However, the minimum number of IR sensors attached to irrigation pipes that would be needed per acre, for example, to estimate ant densities with an acceptable level of accuracy (e.g., ≥85%), is not known. Furthermore, the distribution pattern (e.g., random, zig-zag, or checkerboard) of IR sensors on irrigation lines across rows of trees in a 1 acre block, for example, is also unknown. These 2 additional factors, minimum sensor number per acre and placement patterns, can be resolved with additional fieldwork and appropriate statistical analyses and data modeling.

The ultimate use of IR sensors will be to automate all aspects of *L. humile* management in citrus orchards, from monitoring to deployment of controls. GPS-tagged IR sensors, deployed at appropriate per acre densities and placement patterns, would relay to cloud-based analytic software hourly counts of ants on irrigation pipes. This pipe count data, using analyses presented here, would be converted to estimates of ant densities foraging in trees. “Treat” or “do not treat” recommendations based on grower customizable action thresholds (e.g., ≥100 ants moving on trunks into tree canopies) would be made using count data specific to orchard sectors being monitored. Ant control treatments, such as biodegradable hydrogel beads (McCalla et al. 2020, Milosavljević et al. 2024), would be precision delivered using GPS data from sensors to guide land drones to areas of orchards needing ant control. To maximize control efficacy and bead longevity, hydrogels could be delivered at night when *L. humile* is foraging (Kistner et al. 2017), temperatures are lower, humidity is higher, and orchard workers are absent, thereby circumventing access restrictions due to reentry intervals. The Internet of Things is making these types of sophisticated, accurate, and reduced labor technologies possible, and *L. humile* control in citrus orchards is ideally suited to automated management as described here.

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## Author contributions

Lin Cong (Formal analysis [equal], Validation [equal], Visualization [equal]), Ivan Milosavljevic (Conceptualization [equal], Investigation [equal], Writing—review & editing [equal]), Yehua Li (Formal analysis [equal], Methodology [equal], Validation [equal], Visualization [equal], Writing—original draft [equal], Writing—review & editing [equal]), Weixin Yao (Conceptualization [equal], Supervision [equal], Writing—review & editing [equal]), and Mark Hoddle (Conceptualization [equal], Funding acquisition [equal], Investigation [equal], Methodology [equal], Project administration [equal], Supervision [equal], Writing—original draft [equal], Writing—review & editing [equal])

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## Data availability

The datasets generated and analyzed for this study are available from Yehua Li (yehuali@ucr.edu) upon reasonable request.

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