Survival Rates of *Phytoseiulus persimilis* via Aerial Drone Application

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Introduction

Two-spotted spider mite (TSSM), *Tetranychus urticae*, is a key agricultural pest that occurs across the United States. TSSM is one of the most damaging pests in strawberry production, more specifically central coast strawberry production (Phillips et al., 2015). California strawberry production accounts for 80% of the fresh market strawberries grown in the US, a $1.2 billion dollar a year industry (Gianessi, 2009). TSSM damage appears as stippling, scaring, and bronzing of leaves with yield loss detectable at all infestation levels as low as one mite per leaflet (Phillips et al., 2015). Cell damage and chlorosis from TSSM feeding leads to reduced photosynthesis on affected plants (Butcher et al., 1987). If left untreated TSSM leads to an average 25% yield loss in plots (Gianessi, 2009). Reproduction in TSSM occurs from early spring until late fall, however in coastal strawberry production (such as San Luis Obispo county) reproduction takes place year round (Gianessi, 2009).

Spider Mite Control

A common method for control of spider mites is the release of the predatory mite *Phytoseiulus persimilis* (Gianessi, 2009). Initially, *P. persimilis* released into strawberry fields became established but did not provide an economic level of control for TSSM (Trumble and Morse, 1993). A later study found that release of *P. persimilis* at rates of 320,000 per acre provided effective control of TSSM, however the cost of $2000/acre proved to not be economically feasible (Trumble and Morse, 1993; Anonymous, 2000). Further research found a balance between a release of 30,000 mites per acre and one to two follow up applications of a selective miticide for effective TSSM control (Trumble, 1993; Anonymous, 1989). The cost of the *P. persimilis* release followed by two miticide sprays was estimated at $441/acre (Morse and Trumble, 1989).

Parabug solutions.

The Parabug solution is a patented tool in which growers can use an augmentative approach to better manage the biological control of agricultural pests. Parabug uses unmanned aerial vehicles (drones) to apply biologicals, granulars, and other agricultural materials to crops. Besides the cost of hourly wages, other factors that make current biocontrol methods expensive are insurance, workman's compensation, and opportunity cost of not using the labor on other essential projects. Parabug could greatly increase the efficiency of applying inoculated material to agricultural crops allowing one person and a drone to accomplish the application in a fraction of the time and at a fraction of the cost compared to traditional application methods. (Bennett, 2017).

There is little research on the use of drones to mechanically apply beneficial insects and biological control agents into the field. Only two companies who have brought this technology into conventional agriculture. One of the companies is Australian based ‘Aerobugs Pty Ltd’; the second being ‘Parabug Solutions’ who is owned and created by Cal Poly graduate Chandler Bennett. Chandler will be assisting us in the experimental design of the project. Our project will specifically be testing predatory mite mortality ratios amongst three separate treatments simulating added mortality over time starting from the shipping process to final mechanical application in the field.

Materials and Methods

A prototype drone was acquired from Parabug, the drone was approximately 17 inches by 17 inches by 8 inches with a mechanized rotating application drum on the bottom. By weight the drone is three pounds in total and has a flight time of 30 minutes. All *P. persimilis* were purchased from Mighty Mites in Salinas, CA and overnight shipped to our San Luis Obispo, CA location; Testing was done the same day shipment was received. The mites arrived in a bottle with a sealed cap, filled with vermiculite to make applying the mites easier. The data collected were the number of mites from each sample that were dead or alive. These numbers were put into a ratio (# dead mites / # alive mites) for later statistical analysis. A threshold was established that if the number of mites counted was under 10 that repetition would be invalid and be retested. Before each round of testing the bottle containing the mites was rolled for approximately 10 seconds to ensure even distribution of *P. persimilis* throughout the bottle. Mites were counted immediately after each round of testing under a microscope. The three treatments were: control, mechanical drum roll, and drone drop.

Control, the bottle containing *P. persimilis* was shaken two times into a petri dish coated around the top edges with petroleum jelly to prevent mites from escaping.

Mechanical drum roll, holes in the drum were taped to not allow mites to escape. A portion of the inoculated material was poured into the drum. The drum was rolled at approximately 15 revolutions per minute, for three minutes each replication and then was stopped and mites were counted with the same method as the control.

Drone drop, three yellow sticky cards were mounted on stakes and spaced five feet apart in an even row. The drone was flown down the row of sticky cards approximately ten feet from the ground and seven feet above the top of the sticky cards. The drum speed was set at the same revolutions as the drum roll test (15 revolutions per minute).

Statistical analysis and graphs were generated in Microsoft excel. Three one-way fixed effects analysis of variance (One-factor ANOVA) model were applied to the data sets to test the null hypothesis: there was no significant differences in mortality ratio between each of the three treatments (control vs mechanical drum roll, mechanical drum roll vs drone drop, and drone drop vs control). When alpha was below .05 a Tukey HSD test was used to further compare data.

Results

The experiment was conducted to the parameters with no unexpected errors occuring. All treatments were analyzed using a single factor ANOVA. The control mortality ratio of *P. persimilis* was not significantly different than the mechanical drum roll mortality ratio according to a single factor ANOVA test (Table 1). We failed to reject the null hypothesis that the mortality ratio would be the same between treatments (P= 0.746 α=0.05).

Table 1. A single factor analysis of variance for mortality ratio (#mites dead/ #mites alive) of *Phytoseiulus persimilis* mites, control vs. mechanical drum roll (n=8).

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000901	1	0.000901	0.108832	0.746362	4.60011
Within Groups	0.115915	14	0.00828			
Total	0.116816	15				

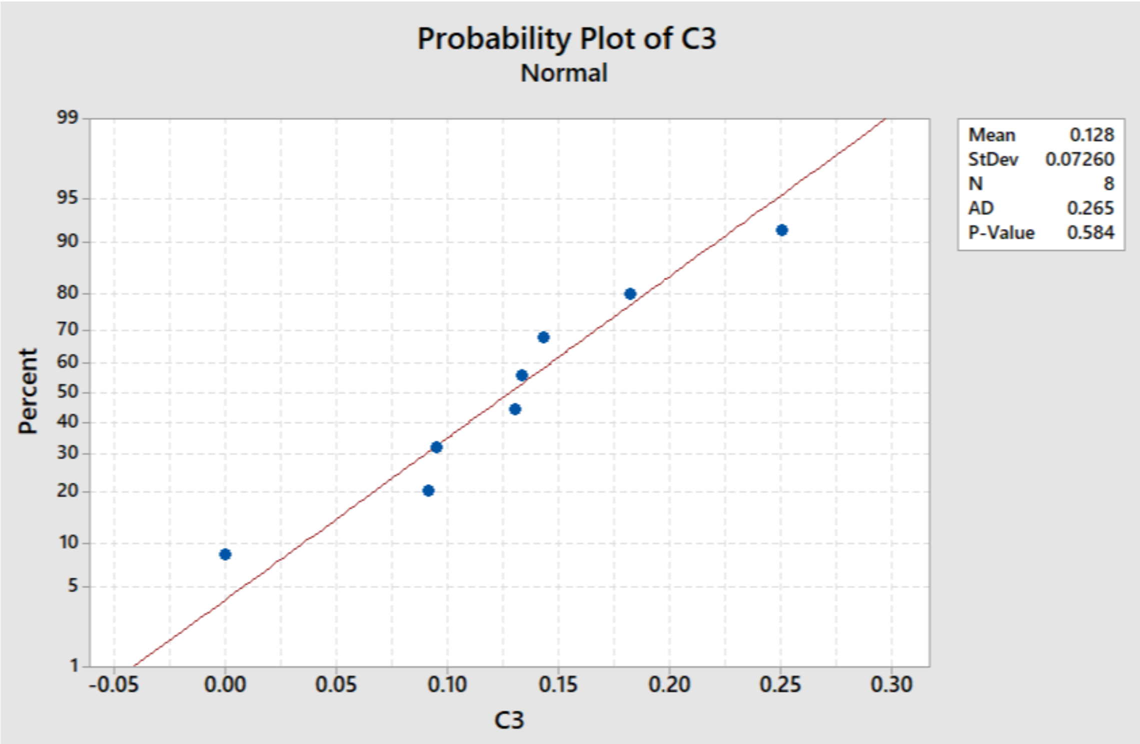

Figure 1. Q-Q Plot for mite mortality ratios (#dead mites /#alive mites) residuals of *P. Persimilis* for the control treatment (n=8).

The mechanical drum roll mortality ratio of *P. persimilis* was not significantly different than the drone drop mortality ratio according to a single factor ANOVA test. We failed to reject the null hypothesis that the mortality ratio would be the same between treatments (*P*= 0.895 α=0.05).

Table 2. A single factor analysis of variance for mortality ratio (#mites dead/ #mites alive) of *Phytoseiulus persimilis* mites, mechanical drum roll vs drone drop (n=8).

ANOVA

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000142	1	0.000142	0.018063	0.895	4.60011
Within Groups	0.109995	14	0.007857			
Total	0.110136	15				

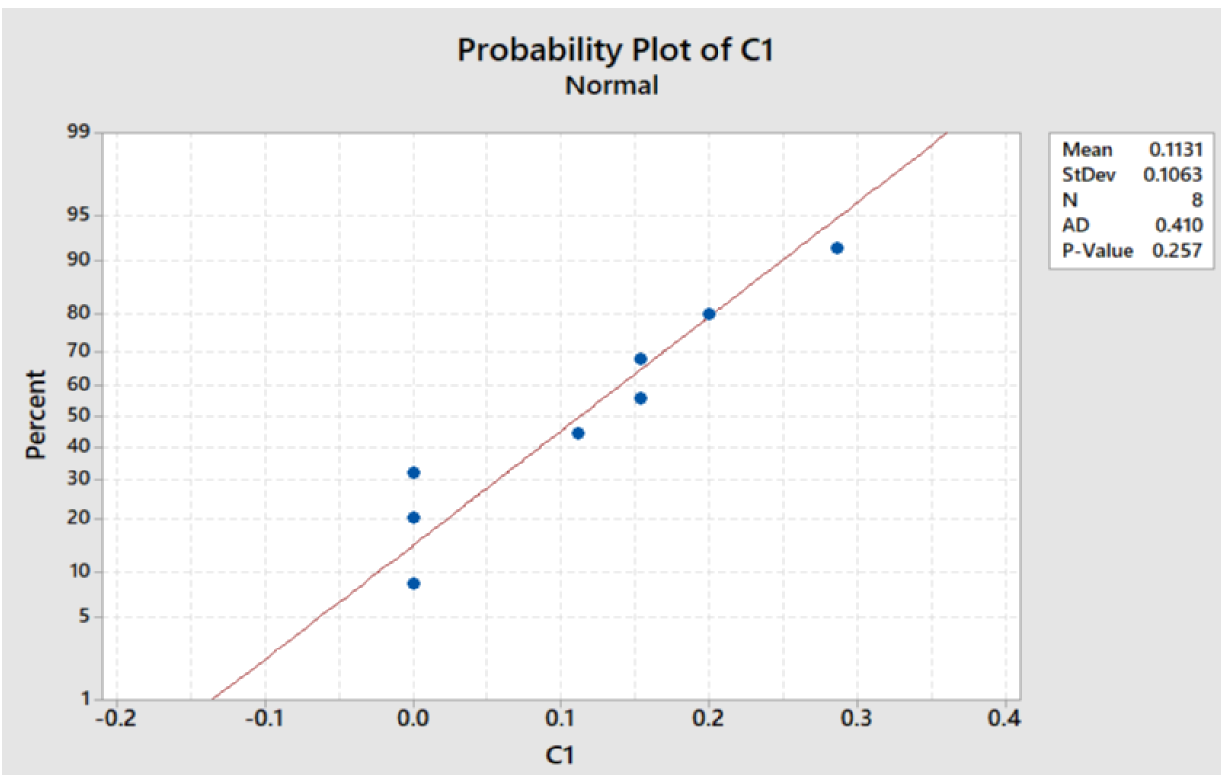

Figure 2. Q-Q Plot for mite mortality ratios (#dead mites /#alive mites) residuals of *P. persimilis* for the mechanical drum roll treatment (n=8).

The drone drop mortality ratio of *P. persimilis* was not significantly different than the control mortality ratio according to a single factor ANOVA test. We failed to reject the null hypothesis that the mortality ratio would be the same between treatments (P= 0.799 α=0.05).

Table 3. A single factor analysis of variance for mortality ratio (#mites dead/ #mites alive) of *Phytoseiulus persimilis* mites, drone drop vs control (n=8).

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	0.000328	1	0.000328	0.067671	0.798545	4.60011
Within Groups	0.067817	14	0.004844			
Total	0.068144	15				

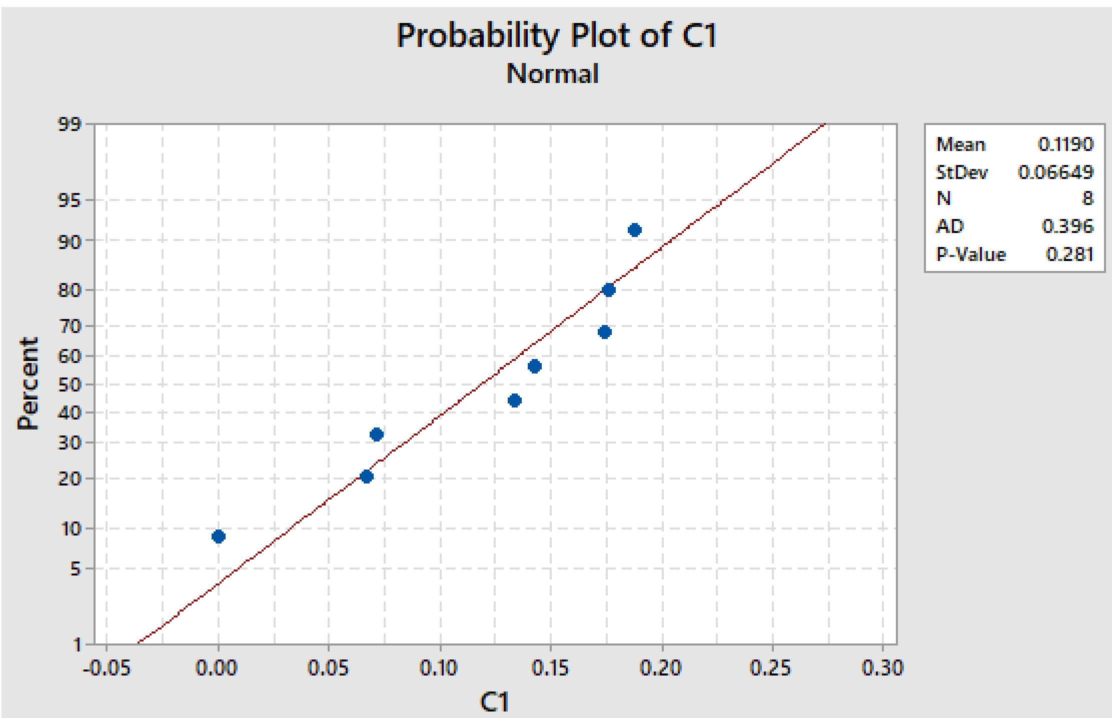

Figure 3. Q-Q Plot for mite mortality ratios (#dead mites /#alive mites) residuals of *P. persimilis* for the drone drop treatment (n=8).

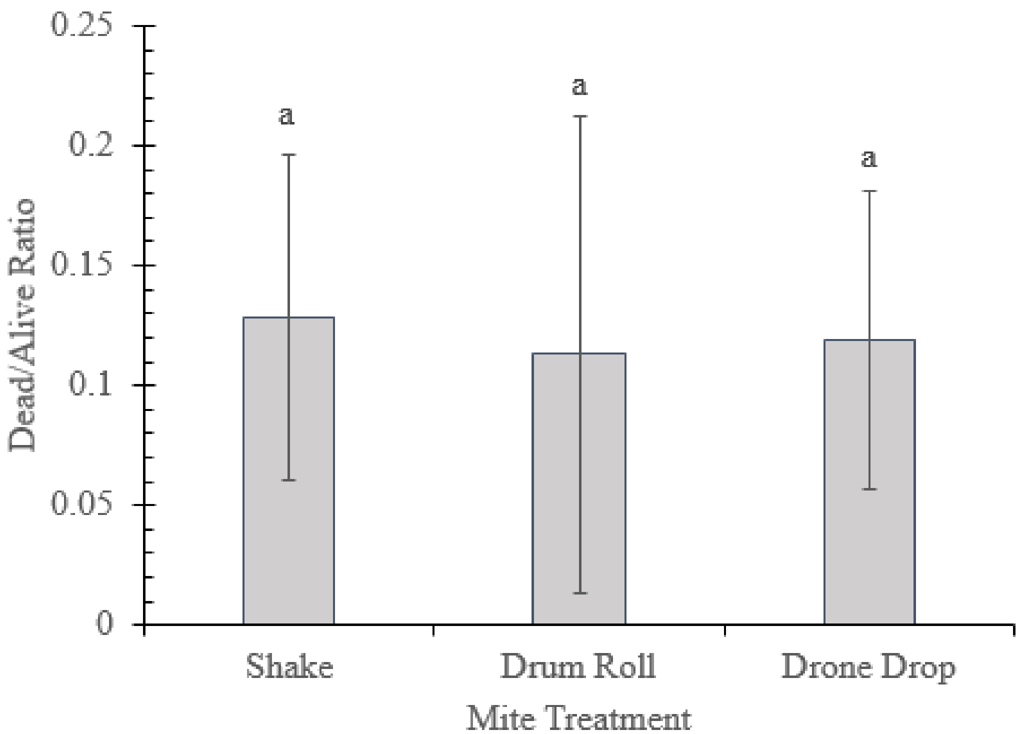

Figure 4. Mite mortality ratios (#dead mites// # alive mites) means for three *P. persimilis* treatments. Means without the same letter are significantly different according to a single factor analysis of variance ($p \leq 0.05$, error ± SE, n=8)

Discussion

This experiment was conducted to observe the added mortality of *P. persimilis* over time, simulating arrival of mites from a supplier all the way to post application. Our data was evaluated using a ratio based system of # dead mites/ #alive mites. One unexpected piece of data was that the mean mortality ratio of our control treatment was higher than the other treatments. However, there was no statistical differences amongst our three treatments (control, mechanical drum roll, and drone drop). We suspect that the higher ratio of dead mites was due to variability between shipments, this shows that growers must know and trust their insect supplier. It is always important to check shipments upon arrival. Our results align with our original hypothesis that a significant number of mites would not be killed through the shipping and application process. Mean mortality ratios of *P. persimilis* should have been additive because as age of mites increases over time, so would overall senescence. This is typically why growers use cultural methods such as cold storage for suspended animation of mites, fragile handling and quick application. If we were to replicate this experiment again, it would have been less difficult for the applicator if the drone was equipped with a GPS system for increased precision. A GPS system would also reduce the factor of operator efficiency and skill into the overall effectiveness of treatments.

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