Effects of Varying Freezing Temperatures on Morality Rate of *Periplaneta americana* (L.) (Blattodea: Blattidae)

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Abstract: *Periplaneta americana* (L.) (Blattodea: Blattidae), commonly known as the American Cockroach, is a common pest in human habitations and structures. Though not a major vector of any pathogens, *P. americana* is known to carry bacterial pathogens such as *Salmonella spp.* (Salmon), which can become a problem in poultry houses. *Salmonella spp.* bacteria is regularly present in the digestive tract of poultry, and when over abundant, it can become a problem when the meat is consumed by humans. With the presence of *P. americana* and other Blattodea species in poultry houses, it can become difficult to manage *Salmonella spp*. Managing pests in the poultry industry can be strenuous and expensive, as many pesticides can be harmful to the poultry if not properly applied. The aim of this experiment was to determine effective lethal freezing temperatures of *P. americana* so methods of eliminating the pest may be developed using cold temperatures. Using varying temperatures, *P. americana* specimens were freezed and death rates were monitored over specific time periods. While freezing the cockroaches below 0˚ C resulted in high mortalities, the intensity of the freeze appeared to be the major contributor to the rate at which the specimens were killed. With this information, future *P. americana* control methods can be set in place not only in poultry houses, but in any infested human structure.

Keywords: *Periplaneta americana, Salmonella spp.*, pest, freezing, exterminate

The American Cockroach, *Periplaneta americana* (L.) (Blattodea: Blattidae), is one of the most loathed pest humans encounter in their homes, businesses, and almost all other man-made buildings. *P. americana* is known to carry numerous bacterial, fungal, and viral infections along with pathogenic parasitic worms (Fathpour et al. 2003). Though not treated as a major vector of any pathogens in small numbers, it is possible *P. americana* could be an issue within poultry houses as they can carry *Salmonella spp.* (Salmon) bacteria, which is regularly present and normal in the gut of poultry (Fathpour et al. 2003, Russell 2021). If *Salmonella* becomes a problem in a poultry house and the bacteria becomes present in the cockroaches that live there, it could be possible that the cockroaches transmit the problem to the next group of poultry that inhabit the house.

Normal treatments to reduce *Salmonella* populations in poultry and poultry products include feed additives, organic acids, and bacteriophages (Van Immerseel et al. 2002, Higgins et al. 2005, Mani-Lopez et al. 2012).

Freezing pests as a means of eliminating them is not commonly practiced although the method is sometimes used to kill bed bug (Hemiptera: Cimicidae) infested clothing and bedding (Olsen et al. 2013). It is known that the intensity of a freeze, the freeze duration, and the cold tolerance and hardiness of the insect have a major impact on an insect's survivability during freezing conditions (Bale 1987). Developing a method of freeze-treating entire facilities for pest insects could provide a safer, cheaper alternative to chemical pesticides.

This study examines how the intensity and duration of a freeze impact the death rates of *P. americana*, possibly determining if freezing is a viable treatment of the pest.

Materials and Methods

Twelve *Periplaneta americana* cockroaches were obtained from a poultry facility in College Station, Texas, and were placed into separate 3"x3"x1.5" clear plastic containers with a lid (Mainstays by Walmart, Bentonville, Arizona). Three of the containers, each containing a single roach, were placed in a 22˚C room. The remaining nine cockroaches were evenly divided into three separate freezers (Hobart Corporation, Troy, Ohio) with temperatures of -23˚C, -9˚C, and 3˚C. The lids of each container were left sitting loosely on top of the containers to ensure air exchange was possible. After one hour, all the containers were removed from their respective freezers and allowed to thaw for one hour in the 22˚C room.

To determine if a specimen was dead, movement, or lack thereof, at the end of each thaw period was observed, and each motionless specimen was dissected to confirm there was no heartbeat. All living cockroaches were returned to the same freezer they were removed from and left there for two hours, thawed for one hour, and then returned to their freezers. The process was repeated for three-, four-, and five-hour cycles, removing dead cockroaches and refreezing the living ones at the end of each thaw period. Once the mortality was recorded for each thaw period, a two-tailed t-test was performed to analyze the data.

Results

Figure 1 is representative of the number of living specimens exposed to each temperature treatment. The depth of freezing temperature was shown to have a major impact on the mortality rate of *P. americana*, with more intense freezes showing faster mortality rate than less intense freezes and non-freezing temperatures. The 22˚C control treatment did not kill any specimens. The single mortality of the 3˚C temperature treatment only occurred after an extended period.

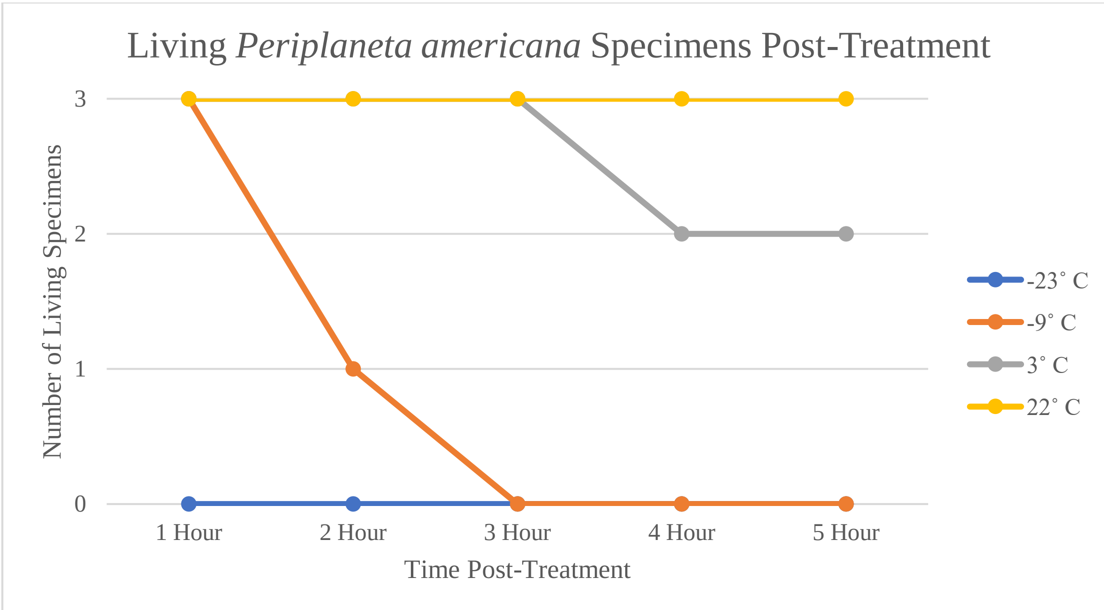

Fig 1. Remaining living specimens for each temperature treatment after each time treatment.

The two below-freezing treatments killed all three replicates but varied significantly in durations of freezing that resulted in a kill (P<0.0258). The -23˚C temperature treatment killed all the cockroaches within the 1-hour time treatment (M=1; SD=0). The -9˚C temperature treatment killed two specimens within the 2-hour time treatment and killed the remaining specimen within the 3-hour time treatment (M=2.33; SD=0.6534). The distribution of the total kills per temperature treatment is represented in Figure 2.

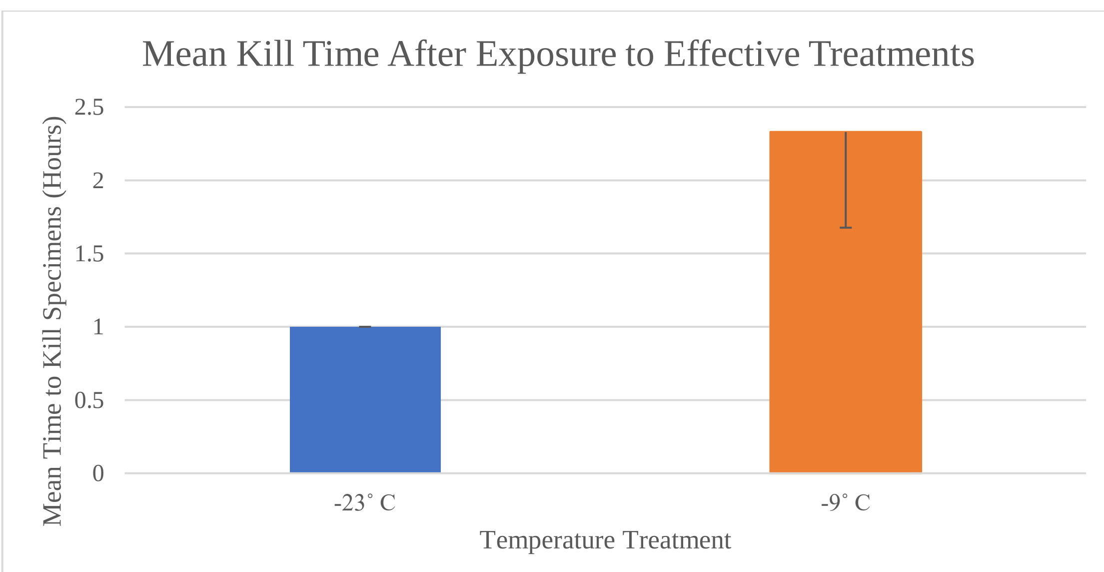

Fig 2. The mean kill times of the two effective treatments. Mortality of P. americana at the -9˚C temperature treatment was significantly higher than the -23˚C temperature treatment (P<0.0258)

Discussion

Periplaneta americana is a pest species commonly found in human habitations and poultry houses. This species is known to carry drug resistant *Salmonella spp.* within poultry houses and *Klebsiella pneumoniae* (Friedlander) within hospitals, along with other bacterial, fungal, and viral pathogens in a variety of other populated locations. Additionally, *P. americana* has the ability to indirectly transmit *Salmonella spp.* bacteria to other cockroaches through their excretions (Fathpour et al. 2003). *Salmonella* bacteria is regularly present in the gut of poultry and only poses a threat to humans when the bacterial load begins to reach extreme population numbers (Russell 2021). If cockroaches within poultry houses are not properly maintained, *Salmonella* treatments have the potential to become ineffective due to the overabundance of *P. americana*. The negligence to implement pest management strategies can consequently allow cockroaches to continue vectoring the bacteria, thus increasing the likelihood of the next round of poultry fostering high loads of *Salmonella*. In the midwestern and northeastern regions of the United States, the exposure of poultry houses to seasonal freezing ambient temperatures overnight, during post-removal and pre-placement of poultry, could prove to be an effective way of killing these cockroaches without pesticide application.

The exact temperature at which *P. americana* freezes to death is unknown. However, in cases where the temperature is not below the minimum lethal temperature (MLT) of the desired specimen, the long duration of exposure to low temperatures has been shown to kill insects due to the freezing of hemolymph (Salt 1936, Hodgson 2016). While there are many methods used to kill insect pests in livestock areas, the process can lead to high expenses and intensive labor to prevent harmful chemicals from affecting livestock. In contrast, freezing insect pests can be an efficient and effective manner of pest control in livestock industries (Salt 1936).

This experiment aimed to narrow the projected MLT of *P. americana*, within reasonable time frames for extermination, to a range of -23ºC to 3 ºC. This information has the potential to be useful in future experiments designed to determine the exact MLT of *P. americana*. Suggestions for replication include using a larger sample population and more repetitions. The implementation of these tactics would allow for increased accuracy of collected data. Additionally, further experimentation may need to be performed to find the MLT of *P. americana* ootheca and eggs.

The discovery of a MLT for *P. americana* can be used to control these pests and limit the risk of transmission of the harbored pathogens. The issuance of this method, when proven to be effective within a suggested time frame of a single night, could be used to develop cold-temperature treatments of homes, hospitals, and poultry houses. This treatment method could be especially useful in areas that experience consistent freezing temperatures throughout specific seasons.

This experiment provides a baseline for finding the exact MLT for *P. americana* regarding the purpose of extermination where pathogens are present in pests. While this data is not an exact representation of the MLT, it could be used in practice when ensured that *P. americana* pests are exposed to temperatures at or below -9 ºC for approximately 2.5 hours. This protocol resembles the standard winter season in the poultry-producing midwestern United States (NOAA 2017).

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