A Bioassay of Insecticidal Paints Containing Pyrethroids, Chlorfenapyr, and/or Growth Regulators for Bed Bug (*Cimex* *lectularius*) Treatment

Abstract

We conducted a simple bioassay by exposing bed bugs (4th and 5th instars) to a petri dish painted with either a control paint or a paint containing Pyrethroid and a growth regulator () for 1, 3, 6, and 24 hours. A third paint containing chlorfenapyr and a pyrethroid paint was also tested with 24 hours of exposure. After exposure, insects were placed in individual tubes and were observed weekly for 1 month. After a month, the remaining insects were fed and were observed to see if they could molt and lay eggs. We repeated the assay at 1 day, 90 days, and 180 days post painting. Though there is variation between each repetition in the effectiveness, the two pesticidal paints killed more insects than the controls. Typically, the 5A-IGR strain killed more insects than the control insects.  The efficacy of these paints was maintained even after 180 days after painting and up to a year as our pilot demonstrated. With this efficacy, we also tested if the insects could detect the pesticide by placing bed bugs on a petri dish painted with both the control and 5A-IGR paint and to see if bed bugs spent a disproportionate amount of time on the control side.  Bed bugs spend -% more time on X side [sign]. Thus with efficacious treatment and \_ detection, this product sh/could \_\_\_ be a part of the solution to control bed bug infestations.

Introduction

Those in health care policy often refer to the idea of the “iron triangle” [ref?.] At the corners of the triangle lie cost, quality, and access. Each of these three traits opposes the other, as if connected by unbending iron rods. If you increase the quality of care, you will be limiting access to such care and/or increase the cost. This concept similarly applies to vector control. Professional pesticide treatments for bed bugs often cost around one thousand dollars[ref a]. This sum often prevents residents from seeking rigorous and hopefully safer treatments licensed pesticide applicators can offer. Cost barriers lead residents to perform their own treatments.  Often these treatments can be dangerous and ineffective. Using foggers, cleaning their home with gasoline, and dosing themselves with alcohol or pesticide have each been documented[ref b] and in some cases have lead to injury, fires, property damage, and even death[ref c and d]. Thus, the high costs of quality care reduces such access forcing residents to pursue low quality, potentially dangerous yet accessible, cheaper care.

Quality, or in our case, the efficacy and comparative effectiveness of professional and self-treatments has not been well studied, especially in the face of growing insecticide resistance [ref.]. In a survey conducted in 2013, there was not a significant difference in the success of professional and do-it-yourself treatments despite the difference in costs[ref b]].  Similar effectiveness may be due to a general lack of efficacy of the pesticides used today [ref]. Many of the insecticides that are currently on the market for both homeowners and professional applicators may not be effective for two reasons. First, the insects have gained resistance to the pesticides[ref e]. Secondly, the method of application strongly influences the pesticide's effectiveness[ref].

To address both concerns of resistance and effectiveness, Inesfly has developed specialized paints that allow for the release of pesticides embedded in microcapsules that burst when insects walk across them.  This application has several potential benefits. Firstly, it could be a do-it-yourself treatment, which would be significantly cheaper than hiring a pesticide applicator. Secondly, it may limit harmful exposures to the pesticide. People would not be spraying pesticide indiscriminately, but would instead paint the corners and crevices along their walls.  Further, it allows for long term control.  Many pesticides and other treatment methods such as heat have no residual capacity meaning they only kill at the time of treatment, so a new infestation can begin if a few insects were not killed or later reintroduced. This paint, however, as we demonstrate maintains efficacy for at least 180 days, and potentially longer than a year as our pilot demonstrated.

Two formulations have been created. The first is 5A-IGR, which is comprised of X ,a growth regulatory, Y, a pyrethroid, and;  Z. The growth regulator functions by serving as an endocrine disruptor that disrupts development. The pyrethorid acts as a neurotoxin. The second  formation is Clorofenapyr and is comprised of Clorofenapyr and Y.  Clorofenapyr is a relatively new pesticide that functions by first being metabolized into an active form that then embeds into the mitochondrial membrane and disrupts the formation of ATP. The benefit to chlorofenapry is two fold. First, unlike pyrethroids, the insects have not gained resistance.  Secondly, it is metabolicly activated by cytochrome enzymes. More of these enzymes are produced in insects with pesticide resistance, so in such insects it may have a greater effect as the active form will be created more quickly. However, being metabolically regulated means that the pesticide’s efficacy is limited by the insect’s metabolism, which is strongly correlated with temperature, with efficacy reaching only adequate levels above X degrees C in trials with mosquitos[ref.].  The lethality also has to compete with the increased fertility and activity of bugs at these higher temperatures.

In this study, we tested the efficacy of these paints in a bioassay by placing bugs on a painted petri dish for various increments of time and observing the insects survival for one month.  We the transfered the remaining insects into jars and fed them to observe differences in development and fertility. We then determined if bed bugs could detect the pesticide by recording movements of bed bugs on petri dishes with both control and 5A-IGR paint.

Methods and Materials

Insecticidal Paint

Three vinyl, aqueous based paint formulations were used.  5A-IGR is a vinyl paint with an aqueous base. It contains alphacypermethrin(0.7%), d-allethrin(1.0%), and pyripoxyfen(0.063%).  The chlorfenapyr formulation contains chlorfenapyr(1.5%) and pyripoxyfen(0.063%). A control formulation without pesticide was also used.  The active ingredients reside in CaCO+ resin microcapsules.  The microcapsules allow for the gradual release of insecticides, which presumably increases duration of effectiveness and reduces the unwanted toxicity of the insecticides ([Amelotti et al. 2009](http://onlinelibrary.wiley.com/doi/10.1111/j.1948-7134.2013.12003.x/full" \l "b2)). The paint was applied to Xcm X-plates following the dosage prescribed by the manufacturer of 10 m2 per liter of paint.  The paint was spread as evenly as possible over each quadrant using cotton swabs. The difference between the initial and final weight of the dish was used to determine the correct amount of paint applied. The paint was allowed to dry in the fume hood.

Insect Colonies

The study was conducted at the University of Pennsylvania (Philadelphia, PA) between September 11, 2015 and April 5, 2016. Bed bugs from the ECL-05 strain were reared in glass jars nested inside a reptile incubator. The temperature and humidity of the enclosure throughout the experiment was maintained between () and  () and recorded at each weekly observation.   The [ECL-05 strain](http://www.sciencedirect.com/science/article/pii/S0022191009001516) was created by EcoLabs from insects collected from commercial properties in Minnesota, Wisconsin, Florida and New Jersey. We obtained our colony from Pennsylvania State University. The colonies were maintained in glass rearing jars with a PVC lid with a hole covered by organza fabric to allow for ventilation and feeding through an artificial feeding apparatus similar to the one described by Montes et al. [ref. ]  Expired Whole blood was obtained from the Hospital of the University of Pennsylvania (Philadelphia, PA). Due to a shortage, blood was obtained from Biological Specialty Corporation (Colmar, PA) on for the 180 day study. Both suppliers used whole blood with CPDA-1 as the anticoagulant.

Bioassays

Fourth and fifth instars were collected and placed in a clean rearing jar. They were fed on an artificial feeding apparatus ( Montes et al. [ref. ])  for 90 minutes one week prior to exposure to the pesticide. Bed bugs were exposed to pesticide 1 day, 90 days, and 180 days after the  petri dishes were painted.  12 bed bugs were placed on each quadrant, except for the 180 day trial were 11 were placed due to high mortality of the insects collected and fed a week prior. There were four X-plates for the 5A-IGR and control treatment groups.  Bed bugs were placed in each quadrant and were exposed for either 1, 3, 6 or 24 hours.  Each X-plate had a quadrant randomly assigned to each time.  After the designated exposure time, bed bugs were placed into 1.5mL microcentrofuge tubes.  A push pin was used to pop three holes to allow for air to enter the tube. Slips of white printer paper were then added to tubes to provide a perch for the insects.  Once all insects had been removed from the pesticide, each insect was observed.  Insects were determined to be either alive, knockdown or dead.  Alive insects were those insects that hand unhindered movement when the observer breathed into the tube or gently prodded the insects with forceps. Knockdown insects had obvious impairment that could include twitching, uncoordinated leg movements, or a lack of movement.   When bed bugs lacked any visible movement, the bed bugs was removed from the tube and the paper was placed against the insect to see if the insect had reflexes to grasp the paper.[ref.]  When the insect failed this test and no further movement was detected it was determined to be dead and placed back into the tube. This paper test was repeated until an insect was determined to be dead for at least three observations or the insect had missing parts. The initial life stage, any molting events, and the number of eggs laid and hatched were also recorded for each insect. Observation was repeated once a week for 4 weeks. After 4 weeks, the remaining bugs for each treatment group were placed into the same jar and fed for 90 min.  For the 1 day and 90 day repetitions, the following week the stage of each insect was recorded. The bugs were returned to the jar and recounted the following week.  For the 180 day study, the insects were counted the day after feeding to determine how many insects fed.

Repellency Experiments:

Data Analysis

Results

Discussion

Figure Legends