THESIS

STUDIES ON THE MANAGEMENT OF THE SWALLOW BUG, *OECIACUS VICARIUS* HORVATH (HEMIPTERA: CIMICIDAE) AND SURVIVAL OFF ITS AVIAN HOST

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ABSTRACT

STUDIES ON THE MANAGEMENT OF THE SWALLOW BUG, *OECIACUS VICARIUS* HORVATH (HEMIPTERA: CIMICIDAE) AND SURVIVAL OFF ITS AVIAN HOST

The swallow bug, *Oeciacus vicarius* Horvath is a common ectoparasite primarily associated with cliff swallows, *Petrochelidon pyrrhonota* (Vieillot). When the mud nests of the cliff swallows are constructed on homes and businesses, swallow bugs often readily enter attics or livable space after the host birds migrate and can cause serious episodes where they may bite humans. To better manage problem situations with swallow bugs a series of studies were conducted to determine the survival of swallow bugs in the absence of their avian host and to evaluate potential methods to monitor and control swallow bugs that do enter buildings.

Swallow nests were collected in 2014 and 2015 immediately after nest abandonment and the nest contents sampled periodically for arthropods. Highest numbers of swallow bugs were found in the first sample dates, immediately after collection, averaging 269 swallow bugs/nest in 2014 and 297 swallow bugs/nest in 2015. Numbers of swallow bugs recovered declined sharply in later samples, with reductions at six months of 97.4% of the adults and 96.7% of the nymphs in the 2014 study, and reductions of 81.9% of the adults and 73.7% of the nymphs died in the 2015 study. At 12 months following collection, numbers of adults and nymphs had declined 99% and 98.3% in the 2014 study and 91.7% and 96.1% in the 2015 study. Other notable arthropods recovered from nests included the dermestid *Trogoderma simplex* Jayne, immature salticid spiders, and the bird flea *Ceratophyllus petrochelidoni* Wagner.

Four traps were evaluated for their ability to capture swallow bugs in an arena test with an introduced swallow bug: a sticky card trap with no attractant (CatchMaster 288i), a carbon dioxide based trap with a collection cup (Bedbug Beacon), a carbon dioxide and heat trap with a bed bug pheromone on a sticky card (Biocare First Response Bed Bug Monitor), and a bed bug pheromone attractant trap with a collecting cup (SenSci Volcano). None of the traps containing attractants showed evidence that they were able to attract swallow bugs. The CatchMaster 288i and BedBug Beacon traps did work well as a passive monitoring device but both the Biocare First Response Monitor and SenSci Volcano SC caught few swallow bugs either because of trap design that allowed the insects to readily escape or prevented their capture due to poor adhesive properties of the glue. Follow-up studies were conducted to evaluate potential attractants in bioassay choice tests, including heat, carbon dioxide, and odors associated with swallow bugs. None of these traps showed evidence of attraction to swallow bugs, suggesting that swallow bugs may use different cues to located hosts than do bed bugs.

Efficacies of insecticides for control of swallow bugs were tested in laboratory trials. Treatments included Suspend Polyzone (deltamethrin), Talstar Professional (bifenthrin), Onslaught Fastcap (esfenvalerate, prallethrin, piperonyl butoxide), Temprid (imidacloprid, cyfluthrin), and Phantom (chlorfenapyr). All of the pyrethroid containing insecticides showed good ability to kill swallow bugs, typically killing 100 percent of the test insects within one week. Lower mortality was observed with chlorfenapyr.

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CHAPTER I. SURVIVAL OF SWALLOW BUGS IN THE ABSENCE OF THEIR PRIMARY HOST AND ASSOCIATED BIOTA IN CLIFF SWALLOW NESTS

INTRODUCTION

The swallow bug, *Oeciacus vicarius* Horvath is a common ectoparasite of cliff swallows, *Petrochelidon pyrrhonota* (Vieillot), and will incidentally feed on house sparrows, *Passer domesticus* L. and other birds that occupy cliff swallow nests or nest in near vicinity (Myers 1928; Usinger 1966; Loye 1985; Smith & Eads 1978; Brown and Brown 1986; Brown et al. 2009). The highest population of swallow bugs occur once the cliff swallow nests are abandoned for the season (George 1987). Hopla & Loye (1983); Eads et al. (1980) reported that swallow bugs can survive prolonged starvation for multiple years, seeking shelter in cracks and crevices around the nest. Ectoparasitism can force cliff swallows into late nesting which can result in declines of fledgling survival fledglings (Brown et al. 2015).

Cliff swallows normally return to previous nesting sites in late April to early May and begin to reoccupy existing nests or build new nests to raise their brood. Cliff swallows migrate out of Colorado to Mexico, Central America and eastern South America generally in August. Swallow bug adults and some instars have the ability to overwinter and have been known to be transported by the cliff swallows (Foster & Olkowski 1968 and Loye 1985). Brown & Brown (1986) reported that some returning cliff swallows can determine whether nests are infested or not infested with ectoparasites (swallow bugs, bird fleas) and may choose free nests or alternate colony sites yearly. Cliff swallows may breed the following year within another colony to minimize ectoparasitism (Brown & Brown 1992). Cliff swallows are more likely to construct new nests than use previous year nests in large colonies, which is believed to be a behavioral response to ectoparasitic infestations.

Boyd (1951) documented a multitude of different ectoparasites that are found associated with cliff swallows including biting lice (Psocodea: Menopodidae, Philopteridae), fleas (Siphonaptera: Ceratophyllidae), hippoboscid flies (Diptera: Hippoboscidae), mosquitoes (Diptera: Culicidae), black flies (Diptera: Simuliidae), bird biting mesostigmatid mites and both hard (Ixodidae) and soft ticks (Argasidae).

Swallow bugs have been reported to carry the Buggy Creek Virus and possibly transmit West Nile Virus (WNV). Brown et al. (2010) reported that Buggy Creek Virus is present in swallow bugs that were unfed in nature for at least two years, providing additional evidence that swallow bugs can persist for multiple years without a food source.

Swallow bugs can also be important as a significant biting pest of humans. Following migration of nesting birds in summer, many swallow bugs disperse from the vacated nests, and often readily enter attics or wall voids of buildings. Once indoors swallow bugs may bite people.

This study investigates survival of swallow bugs in the absence of an avian host and the composition of the nest biota of cliff swallow mud nests from two sites located along the Front Range of Colorado.

METHODS AND MATERIALS

During 2014 and 2015 swallow bugs used in this study were collected from active cliff swallow nests from sites in Weld County (2014) and Boulder County (2015), Colorado. As cliff swallows are protected under the Migratory Bird Treaty Act and possession of the birds, their parts and the nest requires the uses of a permit (Gorenzel & Salmon 1982). USFWS Permit Number MB38079B-0 was granted for this study that allowed only removal of vacated nests. Swallow bugs used in the 2014 study were collected from cliff swallow nests (Fig. 1.1) removed on 11 August from Good Samaritan at Water Valley Senior Living Center in Windsor, Colorado.

Figure 1.1. Windsor, Colorado location where cliff swallow nests were collected on the Good Samaritan building. Nests were accessed from a 38m articulating boom lift and the nests were approximately 20m from the ground.

Hundreds of nests were often constructed on buildings or beneath bridges, often lined in a row with sidewalls connecting the nests. At the time of the collection the nests were vacant from cliff swallows, which had migrated the first week of August, after a bad storm destroyed numerous nests on the north side of the building. For the study initiated in 2014, 25 formerly occupied nests were collected from the Windsor site and individually bagged. When the nests were collected, an attempt was made to keep each nest intact and separately bagged. However, due to the fragile nature of the mud nests and the shared walls on nests that were congregated together (Fig. 1.2) there was some breakage during collections.

The 2015 collections of cliff swallow nests (Fig. 1.2) were taken on 20 August from beneath a bridge in Longmont, Colorado off Pace Street, just north of 17th Ave (USFWS Permit Number MB-61038B-O). Collections were made after the fledge date and the nests were individually bagged in plastic Ziploc bags (Fig. 1.3). A total of 72 formerly occupied nests were collected in 2015.

Figure 1.2 Longmont, Colorado where 72 cliff swallow nests were collected to get swallow bug*)* samples. The picture is underneath a bridge on Pace Street, just north of 17th Street.

Figure 1.3. Removal of individual cliff swallow nests and placement of the nests in Ziploc bags at the Longmont, Colorado location on 20 August 2015. Upon removal, all nests broke apart.

In both years, after the nests were collected and logged they were stored in a heated room (approximately 21°C) at the Agriculture Research, Development and Education Center (ARDEC) of Colorado State University.

Nests were sampled through use of a Berlese funnel (Neethirajan et al. 2007) which was located in the same room where the nests were stored. For each extraction, a single swallow nests was placed in each funnel and extracted for a minimum of three days (maximum of 8 days) to collect all arthropods. The extracted arthropods were collected and stored in 80 % ethanol for subsequent identification and counting.

Nests in the 2014 were extracted using Berlese funnels on five dates: immediately after collection, at two months, four months, six months, and one year after collection. On each date a total of five nests were extracted. Samples from 2015 were processed using Berlese funnels immediately after collection and monthly for 14 months after the initial collection. For each sampling event five cliff swallow nests extracted. Five nests were extracted immediately after collection and another five nests were sampled every month for 12 months and four nests were sampled at 13 months and three nests were sampled at 14 months.

RESULTS

2014 STUDIES, WINDSOR, COLORADO

The initial Berlese funnel extraction in 2014 (Figure 1.4) yielded a total of 385 adult swallow bugs from the five sampled nests (range, 4-295)and 958 nymphs (range, 4-764). The highest number of swallow bugs extracted included 295 adults and 764 nymphs from one nest. This sample was collected within a few days of the birds leaving the nesting site.

After a two month interval of storage extractions from five nests yielded only nine adults (range, 0-4) and 12 nymphs (range, 0-8). This indicated a sharp decrease in both adult (97.7%) and nymphal (98.7%) survival. Extractions of five nests at four months recovered 12 adults (range, 0-5) and 17 nymphs (range, 1-7), representing a 96.9% (adults) and 98.2% (nymphs) decrease compared to the initial extraction. The six month extractions of five nests recovered ten adults (1-3 range) and 33 nymphs (range, 1-11), representing a 97.4% and 96.7% decrease from the initial extraction date. The last collection at 12 months yielded only 4 adults (range, 0-1) and 16 nymphs (range, 1-8); a 99% and 98.3% decrease in the number of bugs from the initial extraction date (Fig. 1.4).

A few salticid spiders and larvae of the dermestid *Trogoderma simplex* Jayne were also extracted from the nests in 2014.

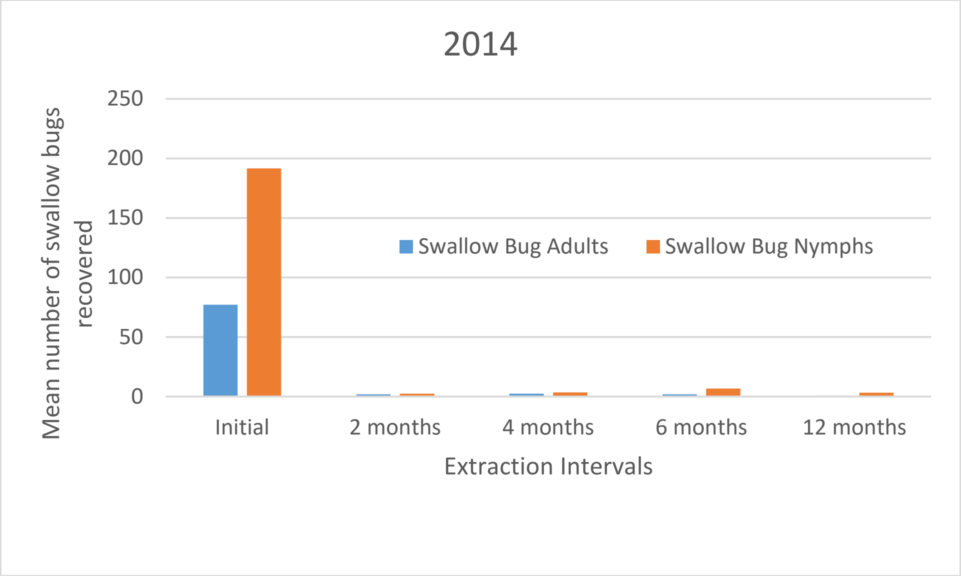

Figure 1.4. Mean numbers of swallow bug adults and nymphs recovered from cliff swallow nests collected in 2014 (Windsor, Colorado). Nests were collected within one week after the nest site was abandoned and collected nests were stored individually in Ziploc bags. On each extraction date, beginning at the date of collection, the arthropods from a subsample of five nests were extracted with a Berlese funnel. The results indicate the mean number of swallow bug adults and nymphs extracted per nest on each date.

2015 STUDIES, LONGMONT COLORADO

Initial collections from the five swallow nests extracted in 2015 (Figure 1.5) included 144 adults (range, 20-40) and 1,340 nymphs (range, 128-478). The highest nest sample included 32 adults and 472 nymphs (504 swallow bugs). Extraction after one month, recovered 96 adults (range, 4-40), and 764 nymphs (range, 60-258), representing a 33.3% and 43% decrease in the number of adults and nymphs, respectively, recovered compared to the initial collections from the nests. Berlese extractions of five nest after two months yielded 63 adults (range 4-20) and 894 nymphs (range 86-287) indicating a 56.3% and 33.3% decrease of adults and nymphs, respectively, compared to initial collections. Extractions of five nests at three months yielded 49 adults (range, 3-21) and 764 nymphs (range, 60-288) representing a 66% and 43% reduction of adults and nymphs compared to the initial collections. Four month extractions yielded 24 adults (range, 0-13) and 330 nymphs (range, 26-173), a reduction of 83.3% (adults) and 75.4% (nymphs) from the initial collections. Five month extractions yielded 41 adults (range, 5-14) and 553 nymphs (range, 26-173), a reduction of 71.5% (adults) and 58.7% (nymphs) from the initial collections. Six month extractions yielded 28 adults (range, 0-10) and 264 nymphs (range 3-88), a reduction of 80.6% (adults) and 80.3% (nymphs) from the initial collections. Seven month extractions yielded 26 adults (range, 2-16) and 353 nymphs (range, 24-160), a reduction of 81.9% (adults) and 73.7% (nymphs) from the initial collections. Eight month extractions yielded 4 adults (range, 0-3) and 96 nymphs (range, 8-35), a reduction of 97.2% (adults) and 92.8% (nymphs) from the initial collections. Nine month extractions yielded 39 adults (range, 0-25) and 177 nymphs (range, 0-102), a reduction of 72.9% (adults) and 86.8% (nymphs) from the initial collections. Ten months extractions yielded 32 adults (range, 2-10) and 163 nymphs (range, 2-48), a reduction of 77.8% (adults) and 87.8% (nymphs) from the initial collections. Eleven month extractions yielded 34 adults (range, 0-30) and 203 nymphs (range, 0-140), a reduction of 76.4% (adults) and 84.9% (nymphs) from the initial collections. Twelve month extractions yielded 12 adults (range, 0-8) and 52 nymphs (range, 2-23), a reduction of 91.7% (adults) and 96.1% (nymphs) from the initial collections. Thirteen month extractions yielded one adult (range, 0-1) and 38 nymphs (range, 5-12), a reduction of 99.3% (adults) and 97.2% (nymphs) from the initial collections. Fourteen month extractions yielded four adults (range, 1-

2) and 49 nymphs (range, 10-22), a reduction of 97.2% (adults) and 96.3% (nymphs) from the initial collections (Fig. 1.5).

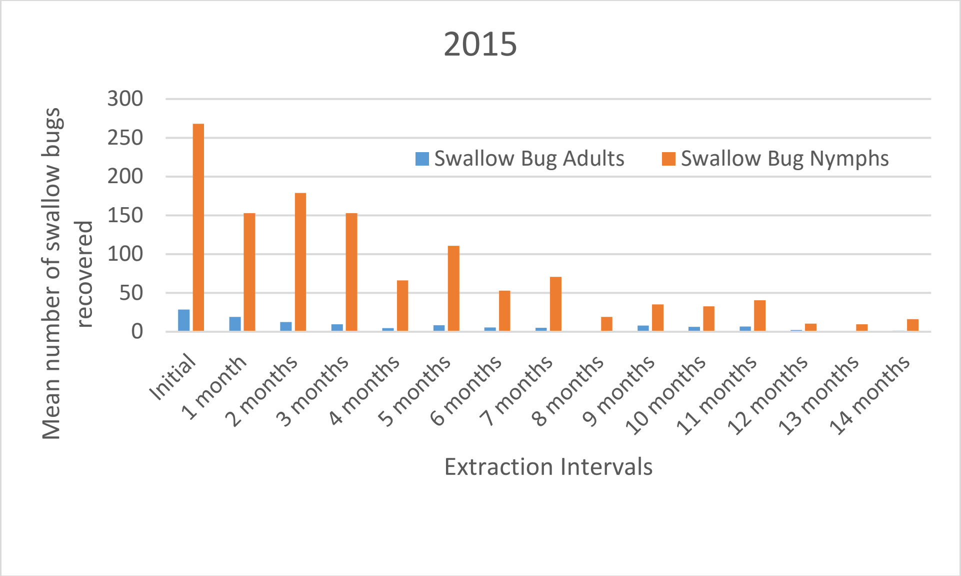

Figure 1.5. Mean number of swallow bug adults and nymphs per nest extracted from cliff swallow nests collected 2015 (Longmont, Colorado). Nests were collected within one week after the nest site was abandoned and collected nests were stored individually in Ziploc bags. On each extraction date, beginning at the date of collection, the arthropods from a subsample of five nests were extracted with a Berlese funnel monthly for fourteen months. The results indicate the mean number of adults and nymphs extracted on each date from the five nests. Mean numbers at the thirteen month extraction was based on a sample of four nests; and the fourteen month extraction was based on a sample of three nests

In 2015 samples, the bird flea *Ceratophyllus petrochelidoni* Wagner were recovered from 17 of the 72 or 24% of nest samples. Again larvae of *T. simplex* were present, higher numbers than in 2015, along with three immature salticid spiders.

Returning cliff swallows returning the next season did not rebuild their nests at the same site and only used the nests that were already built. No new construction of nests was observed in areas of nest removals either at the Windsor or Longmont locations.

DISCUSSION

Study results indicate that the highest number of swallow bugs were present in a nest immediately after fledging and nest abandonment. Nests collected within one week after fledging averaged 269 swallow bugs/nest in 2014 and 297 swallow bugs/nest in 2015. This is substantially higher than reported than the average of 32 swallow bugs/nest reported by Orr & McCallister (1987). High variability was observed in the number of swallow bugs infesting individual nests during the initial surveys ranging from 8-1,059/nest in 2014 and 148-504/nest in 2015 samples.

Swallow bugs have been reported to survive for long periods after nest abandonment following fledging (Foster & Olkowski 1968; Loye 1985; Hopla and Loye 1983; Smith & Eads 1978 and Brown et al. 2010). Both years of Berlese extractions showed a sharp decline in the number of swallow bugs recovered from initial extractions from nests compared to subsequent extractions. These results were similar to those of Loye (1985), who reported that 99% of all swallow bugs died within seven months. Brown et al. (2010) reported that swallow bugs collected directly from the nest did not live more than two years without its host.

In 2014 97% of adults and 97% of nymphs did not survive six months after collection. In 2015, 82% adults and 74% of nymphs apparently died within the same seven month period. The relative mortality observed in both 2014 and 2015 indicated lower mortality over the same six or seven months. Loye (1985) mortality estimates were taken from field sampling during ambient air temperatures. In the present study nests were stored indoors at room temperature and there was some fragmentation of the collected nests.

Hopla & Loye (1983) reported that in field conditions swallow bugs did not live more than three years without a host and that all life stages overwintered. All nymphal stages and adults were reported to overwinter by Foster & Olkowski (1968) and Loye (1985) but it was observed that a majority of the overwintering swallow bugs were adults. In this present study, in both years (2014, 2015), both nymphs and adults were recovered after 12 months, but nymphs were present in highest proportion at this time (4 adults/16 nymphs in 2014, 12 adults/52 nymphs in 2015).

The only other laboratory (vs. field) based study on survival of swallow bugs available is by Loye (1985) who evaluated survival without a blood meal and without a host. This study indicated that swallow bugs did not survive longer than one year under these conditions. In the present study unfed swallow bugs were observed to survive over a year and survival of some continued for as long as fourteen months in 2015, when the study was terminated.

During sampling of the Longmont site in 2015 the bird flea, *Ceratophyllus petrochelidoni* was found to be a common nest associate, present in 24% of all nests sampled. Brown and Brown (1992) reported finding cliff swallow fledglings parasitized by the related *C. celsus* Jordan in southwest Nebraska. These bird fleas are replaced by *C. petrochelidoni* and *C. scopulorum* Holland in the far West (Pilgrim & Galloway 2000). Most of the records of *C. petrochelidoni* are from California (Foster & Olkowski 1968; Schwan, Higgins & Nelson 1983); or Nevada (Nelson 1972). *Ceratophyllus petrochelidoni* was considered “rare in Colorado” by Campos (1971) but the collections at the Longmont study area indicate that this species is not uncommon at least in some sites in Colorado.

Also observed were some acarid mites, midges, and parts of other unidentifiable insects. Immature salticid spiders were also found in some samples, which were the only potential swallow bug predators recovered from nest samples.

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CHAPTER II. SWALLOW BUG TRAPPING TRIALS

INTRODUCTION

A significant human health problem can develop when cliff swallows, *Petrochelidon pyrrhonota* (Vieillot), nest on buildings and subsequently support development of their primary ectoparasite, the swallow bug *Oeciacus vicarius* Horvath. After swallows fledge and abandon nests in summer, the swallow bugs often migrate into living areas of homes where they can bite humans, but humans are considered a dead end host.

The swallow bug is a very common ectoparasite primarily associated with cliff swallows and will incidentally also develop on house sparrow, *Passer domesticus* L., and other birds that may nest in or adjacent to cliff swallow nests (Myers 1928; Brown et al. 2009). Swallow bugs have been reported to carry the Buggy Creek Virus and possibly transmit West Nile Virus (WNV). Brown et al. (2010) reported that Buggy Creek Virus can still be present in swallow bugs that were unfed in nature for at least two years, providing evidence that swallow bugs can persist for multiple years without a food source.

In addition to the human health concerns associated with swallow bugs, these cimicids can also be mistaken for other similar species in Colorado homes, notably bat bugs (*Cimex adjunctus* Barber, *C. pilosellus* Horvath) and the bed bug (*C. lectularius* L.). Since there are considerable differences in the importance of the cimicid bugs to humans proper pest management approaches are required. Furthermore, although the bed bug has received most research attention, particularly focused on control methods (Reinhardt & Siva-Jothy 2007) there has been very little work done with control of either bat bugs or the swallow bug.

For bed bugs there has been a great deal of attention into methods of detection, including traps, protocols for visual inspections, and even use of dogs to determine the presence of bed bugs (Reinhardt & Siva-Jothy 2007; Vaidyanathan & Feldaufer 2013). Trapping for bed bugs has proven to be effective. Interceptor traps used in an IPM (Integrated Pest Management) program were more effective at determining the presence of bed bugs in individual units than visual inspections alone (Wang et al. 2009 B). Anderson et al. (2009) showed that traps using carbon dioxide caught more bed bugs that those traps without carbon dioxide. Wang et al. (2011) tested three bed bug traps including the CDC3000, NightWatch and a home-made dry ice pitfall trap. These authors concluded that bed bugs could be attracted by carbon dioxide (dry ice) pitfall traps in apartments where bed bugs could not be visually detected.

Research conducted by (Legrand et al. 2016) suggested that carbon dioxide in the form of dry ice is the best attractant for bed bugs. Alternative ways of producing carbon dioxide like sugar and yeast monitors in addition with an experimental lure is 7.2 times more effective than climb up traps especially in vacant units. These traps can provide an affordable way of monitoring bed bug activity (Singh et al. 2015). There were no differences between trapping success of traps that had a carbon dioxide cylinder versus a sugar and yeast mixture (Singh et al. 2012). Although all traps, including a dry ice pitfall design (CDC3000) and Night Watch were successful in detecting bed bug presence, the dry ice pit fall trap was most effective and cheaper than the other two active traps (Wang et al. 2011). Pitfall traps baited with heat or carbon dioxide are effective tools for determining bed bug presence with a small population. Carbon dioxide outcompeted heat, but both will attract bed bugs (Wang et al. 2009 A). Trapping efforts were primarily examined different forms of carbon dioxide and heat, Aak et al. (2014) indicated that it takes three days for bed bugs to acclimate to an arena environment and five days outside of an arena when the bed bugs are exposed to human stimuli. They also suggest that an effective and affordable option is to use a pitfall trap with a chemical lure and a sugar and yeast mixture. Gries et al. (2015) discovered a bed bug aggregation pheromone that could be used as an attractant in affordable bed bug traps. Weeks et al. (2011) has shown how important knowledge of the chemical ecology of bed bugs has led to the use of pheromone attractants for bed bug pest management.

Presently, there are a great many commercial devices being marketed for monitoring for bed bugs ranging from passive monitors that mimic harborage locations to active monitors that have attractants or lures. However, none of the monitoring approaches available for bed bugs have been evaluated for use in detection of other cimicids that may occur within living areas. The purpose of this study was to evaluate some existing traps marketed for bed bugs and determine how well these devices trap swallow bugs. Selections were made of traps that had included an attractant such as carbon dioxide, heat, or bed bug pheromones.

MATERIALS AND METHODS

SWALLOW BUG TRAPPING TRAIL – PILOT FIELD STUDY

A pilot field study was conducted to assess various traps used to capture bed bugs for their ability to capture swallow bugs. Traps included a sticky card trap with no attractants (CatchMaster 288i, CatchMasterGlueBoards.com, Winder, Georgia), a carbon dioxide based trap with a collection cup (Bedbug Beacon^{TM}, Nuvenco Inc., 2518 Midpoint Drive, Fort Collins, Colorado), a carbon dioxide and heat trap with a bed bug pheromone on a sticky card (Biocare First Response Bed Bug Monitor, SpringStar Inc., Woodlinville, Washington), and a bed bug pheromone lure trap with a collecting cup (SenSci Volcano^{TM}, Bed Bug Central, Lawrenceville, New Jersey) (Fig. 2.1).

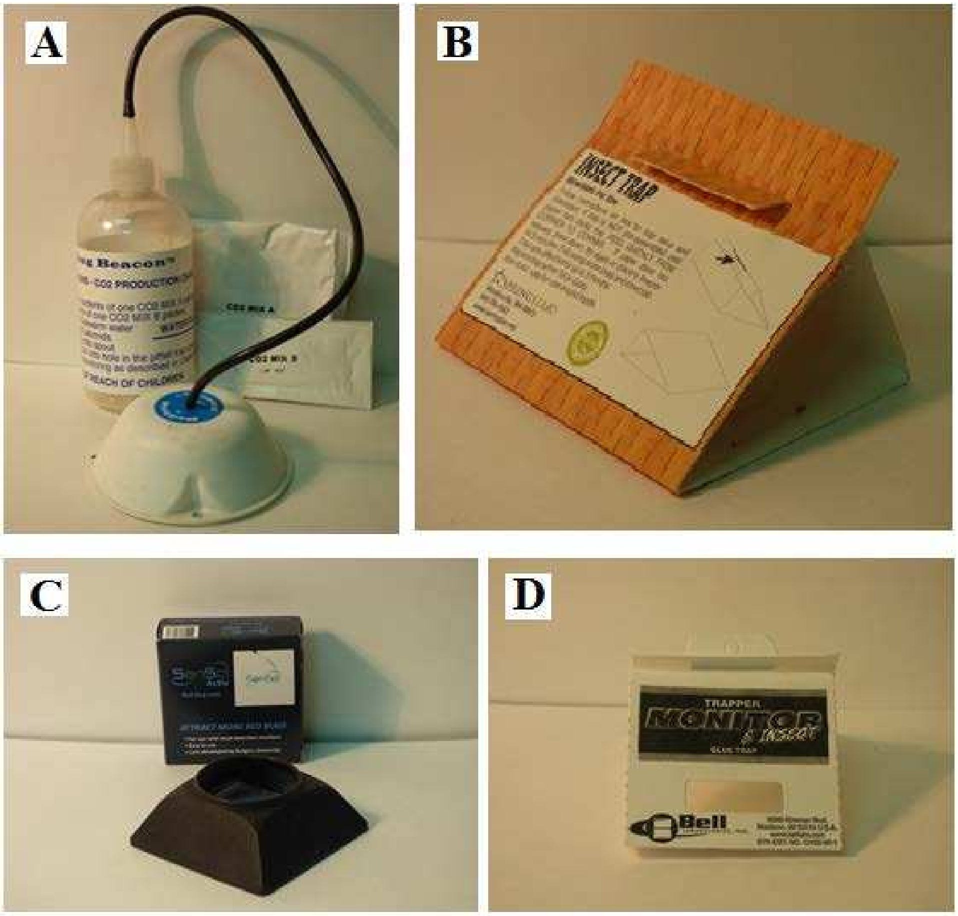

Figure 2.1. Four different traps on the market that are designed to trap bed bugs. These four traps are used to test the efficacy on swallow bugs, *Oeciacus vicarius* (Horvath). A. Bedbug Beacon trap (carbon dioxide attractant), B. First Response Bed Bug Monitor trap (glue pad with carbon dioxide with heat and pheromone, C. SenSci Volcano trap (pheromone), and D. Catch Master (control) sticky glue pad trap (no attractant).

The pilot study (trapping trials) was conducted at a cliff swallow nest site located underneath an overpass on Pace Street in Longmont, Colorado. Beneath the overpass small platforms 20cm x 20cm were installed every 7.6m along the wall and 61cm below the nesting area to fashion traps to the wall for the study. Traps were erected 1 August 2015 and removed 8

August 2015 (Fig. 2.2). The results of this field trial were poor with only a few swallow bugs captured during the seven days of the trial and it was subsequently discontinued.

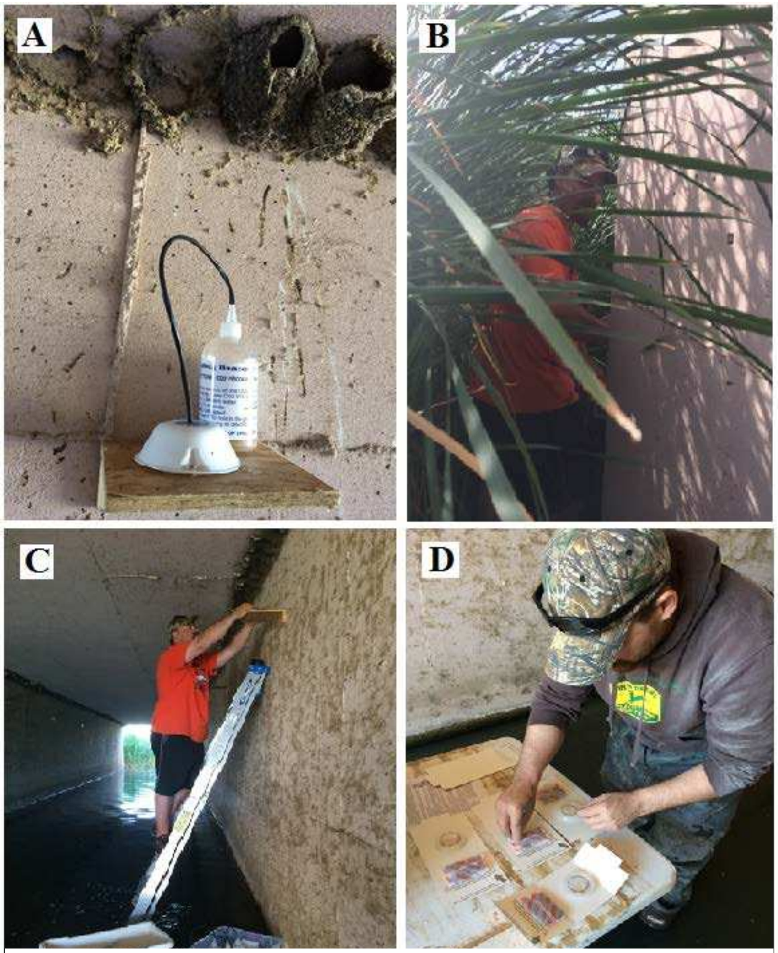

Figure 2.2. Steps involved in the trap deployment underneath the bridge in Longmont, Colorado. A. Bedbug Beacon trap deployed underneath the recently vacated cliff swallow nests. B. Gaining access to site where cliff swallow nests were constructed. C. Setting the traps underneath the bridge. D. Setting up the Biocare First Response Bed Bug Monitor traps with a combination of a pheromone, heat pack and carbon dioxide cylinder.

SWALLOW BUG TRAPPING TRIALS – ARENA TESTING

The four traps included in the pilot field study (CatchMaster 288i, BedBug Beacon, Biocare First Response Bed Bug Monitor, SenSci Volcano) (Fig. 2.1) were tested in a series of arena trials to compare swallow bug capture efficacy. Arenas consisted of clear Sterilite^{R}

containers that were 58.4cm long, 41.3cm wide and 15.4cm high, which was sufficient to prevent released swallow bugs from escaping the arena. The design of the plastic containers also included a depression of about 0.2cm which ringed the perimeter of the arena floor. As this depression was steep enough to potentially impede free movement of the insects in the arena, the depression was filled with sand to level out the arena bottom.

Six identical arenas were established and during each trial run single traps of each design were placed in each of the four corners (Fig. 2.3). The arenas were placed on table tops inside a room with temperatures that ranged from 21.1 - 26.7°C. No artificial light was provided but the room had windows that allowed some lighting of the area during the day light period.

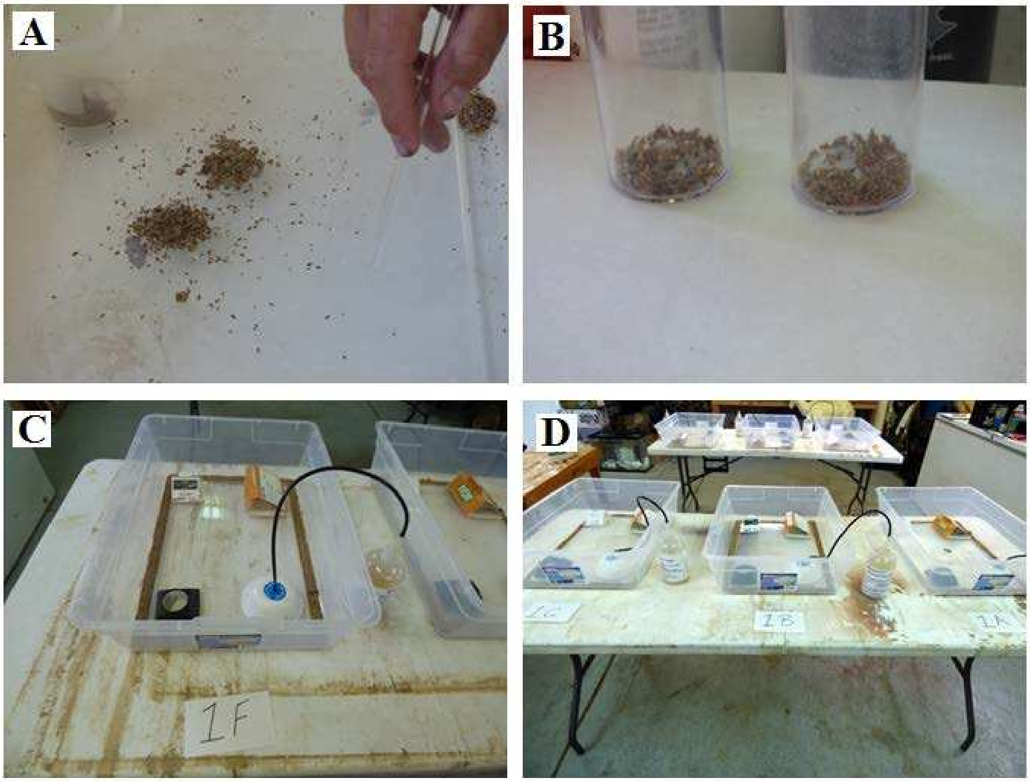

Figure 2.3. The arena trapping trials. A. Collection of swallow bugs *Oeciacus vicarius* (Horvath) with an aspirator. B. Vials full of 200 swallow bugs awaiting placement within the arenas. C. An arena trial showing the plastic container with sand surrounding the outer depression. Also the three active traps and one passive monitor that is acting as a control. D. All six arenas of a trial in progress.

Swallow bugs used in these trials consisted of both adults and different nymph instars. They were collected 1 August 2015 from cliff swallow nests located beneath a bridge in Longmont, Colorado. To extract the swallow bugs for these trials nests were put through a Berlese funnel (Neethirajan et al. 2007), collecting them in dry cups. Once the swallow bugs were in the dry cups they were dumped into a plastic Sterilite container, size not dependent and the swallow bugs were released. Once released the insects were collected and counted with an aspirator. Two hundred swallow bugs including both nymphs and adults were then put in collection cups awaiting their placement into the arenas.

The first trial (1A) was conducted 2 August 2015. After all traps were set up and allowed to sit for 30 minutes, 200 live swallow bugs (combination of adults and different nymph instars) were placed into the middle of the six arenas. Every 24 hours the number of insects present in each trap was recorded and trapping records were kept for seven consecutive days.

A second trial (1B) was repeated on 12 August 2015 with another six arenas using 200 swallow bugs (combination of adults and different nymph instars) per arena. Again the testing results were tabulated every 24 hours and for seven consecutive days.

Experimental design was modified for a third trial to test that swallow bugs were not using the traps simply as a harborage. In this trial the arrangement of traps in the arena was similar, but this time none of the traps were installed with their respective attractants (carbon dioxide, heat, pheromones). Again the number of swallow bugs found in each trap was recorded every 24 hours for seven days. Each arena had the same 200 swallow bugs (combination of adults and different nymph instars) and the experiment began 19 September 2015.

In a fourth arena trial a further modification of the design was added to include a harborage area at the release point of the trial. The arrangement of the four traps was similar to the previous trials, but in this trial, swallow bugs were first placed in a small cup with a 2.5cm x 2.5cm piece of cardboard. The cardboard served as a harborage into which the swallow bugs settled either inside or on the exterior and the cardboard was then placed in the center of the arena. A total of 25 swallow bugs (combination of adults and different nymph instars) were used in two replications of this study. The number of insects present in traps was checked daily for one week. Since very few (two) swallow bugs left the harborage site the study was discontinued.

RESULTS

In the first trial (Fig. 2.4) captures were monitored for seven days. The CatchMaster 288i trap serving as the control with no attractants captured 98 swallow bug (8.2%) on day one, 140 swallow bugs (11.7%) after day two, 164 swallow bugs (13.7%) after day three, 183 swallow bugs (15.3%) after day four, 198 swallow bugs (16.5%) after day five, 199 swallow bugs (16.6%) after day six, and 204 swallow bugs (17%) after day seven. The Biocare First Response trap captured 59 swallow bugs (4.9%) on day one, 65 swallow bugs (5.4%) by day two, 71 swallow bugs (5.9%) day three, 72 swallow bugs (6%) day four, 75 swallow bugs (6.3%) day five, 78 swallow bugs (6.5%) day six and 78 swallow bugs (6.5%) after seven days. The SenSci Volcano trap captured 52 swallow bugs (4.3%) day one, 72 swallow bugs (6%) day two, 80 swallow bugs (6.7%) day three, 90 swallow bugs (7.5%) day four, 94 swallow bugs (7.8%) day five, 100 swallow bugs (8.3%) day six and 107 swallow bugs (8.9%) after seven days. The Bed Bug Beacon captured 160 swallow bugs (13.3%) day one, 183 swallow bugs (15.3%) day two, 205 swallow bugs (17.1%) day three, 221 swallow bugs (18.4%) day four, 236 swallow bugs

(19.7%) after day five, 238 swallow bugs (19.8%) day six, and 243 swallow bugs (20.3%) after day seven.

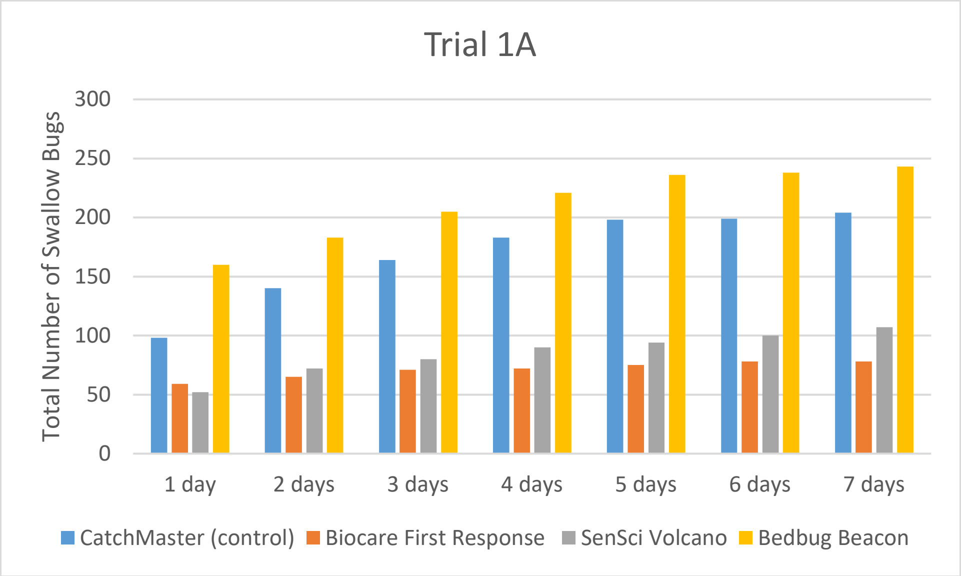

Figure 2.4. Numbers of swallow bugs collected by different traps placed in arena trial settings. Traps included a sticky card trap with no attractant that served as a control (CatchMaster 288i), a carbon dioxide based trap with a collection cup (Bedbug Beacon^{TM}), a carbon dioxide and heat trap with a bed bug pheromone on a sticky card (Biocare First Response Bed Bug Monitor), and a bed bug pheromone lure trap with a collecting cup (SenSci Volcano^{TM}). Trials 1A and 1B were conducted identically. A total of 1200 swallow bugs (200 per replication) were introduced into the arena setting in each trial and the figures indicate the total number captured.

The second trial 1B (Fig. 2.5) was a replicate of the above trial, conducted over the same seven days. The Catchmaster 288i control trap captured 152 swallow bugs (12.7%) day one, 181 swallow bugs (15.1%) day two, 231 swallow bugs (19.3%) day three, 238 swallow bugs (19.8%) day four, 244 swallow bugs (20.3%) day five, 250 swallow bugs, (20.8%) day six and 254 swallow bugs (21.2%) after seven days. The BioCare First Responder trap captured 40 swallow bugs (3.3%) day one, 47 swallow bugs (3.9%) day two, 57 swallow bugs (4.8%) day three, 64 swallow bugs (5.3%) day four, 68 swallow bugs (5.7%) day five, 69 swallow bugs (5.8%) day six and 69 swallow bugs (5.8%) after day seven. The SenSci Volcano captured 39 swallow bugs (3.3%) on day one, 46 swallow bugs (3.8%) by day two, 50 swallow bugs (4.2%) day three, 50 swallow bugs (4.2%) day four, 52 swallow bugs (4.3%) day five, 52 swallow bugs (4.3%) day six and 52 swallow bugs (4.3%) after seven days. The BedBug Beacon captured 151 swallow bugs (12.6%) day one, 194 swallow bugs (16.2%) day two, 260 swallow bugs (21.7%) day three, 283 swallow bugs (23.6%) day four, 297 swallow bugs (24.8%) day five, 303 swallow bugs (25.3%) day six and 306 swallow bugs (25.6%) after seven days.

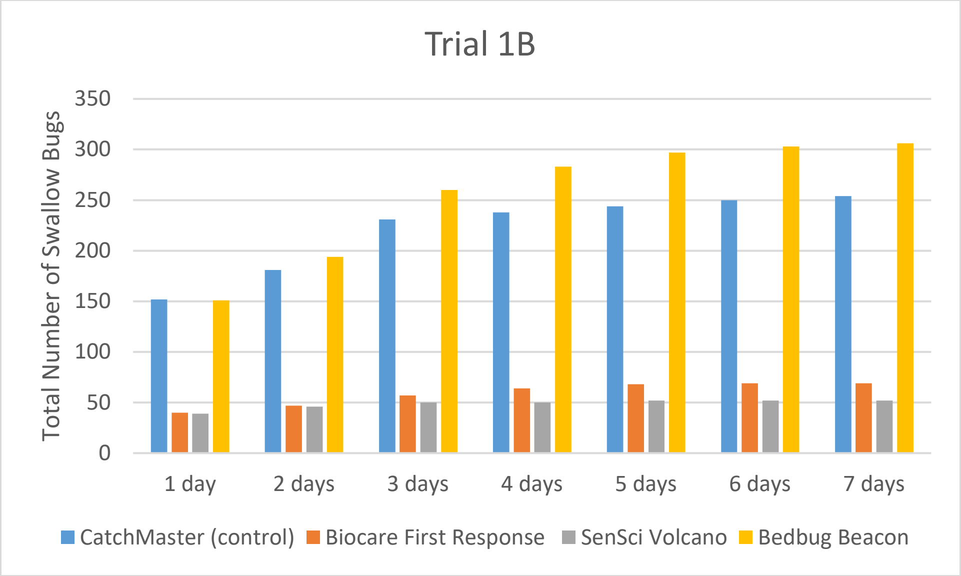

Figure 2.5. Numbers of swallow bugs collected by different traps placed in arena trial settings. Traps included a sticky card trap with no attractant that served as a control (CatchMaster 288i), a carbon dioxide based trap with a collection cup (Bedbug Beacon^{TM}), a carbon dioxide and heat trap with a bed bug pheromone on a sticky card (Biocare First Response Bed Bug Monitor), and a bed bug pheromone lure trap with a collecting cup (SenSci Volcano^{TM}). Trials 1A and 1B were conducted identically. A total of 1200 swallow bugs (200 per replication) were introduced into the arena setting in each trial and the figures indicate the total number captured

In the third trial 1C (Fig. 2.6) the trial was conducted in a similar manner but the attractants (carbon dioxide, heat, and pheromone) were removed. The Catchmaster 288i control captured 148 swallow bugs (12.3%) on day one, 187 swallow bugs (15.6%) by day two, 215 swallow bugs (17.9%) day three, 220 swallow bugs (18.3%) day four, 230 swallow bugs (19.2%) day five, 244 swallow bugs (20.3%) day six and 251 swallow bugs (20.9%) after day seven. The BioCare First Responder trap captured 23 swallow bugs (1.9%) day one, 23 swallow bugs (1.9%) day two, 26 swallow bugs (2.2%) day three, 22 swallow bugs (1.8%) day four, 23 swallow bugs (1.9%) day five, 19 swallow bugs (1.6%) day six and 22 swallow bugs (1.8%) after seven days. The SenSci Volcano captured 30 swallow bugs (2.6%) day one, 27 swallow bugs (2.3%) day two, 36 swallow bugs (3%) day three, 24 insects (2%) day four, 21 swallow bugs (1.6%) day five, 24 swallow bugs (2%) day six and 17 swallow bugs (1.4%) after seven days. The BedBug Beacon captured 120 swallow bugs (10%) day one, 179 swallow bugs (14.9%) day two, 212 swallow bugs (17.7%) day three, 229 swallow bugs (19.1%) day four, 245 swallow bugs (20.4%) day five, 252 swallow bugs (21%) day six and 260 swallow bugs (21.2%) after seven days.

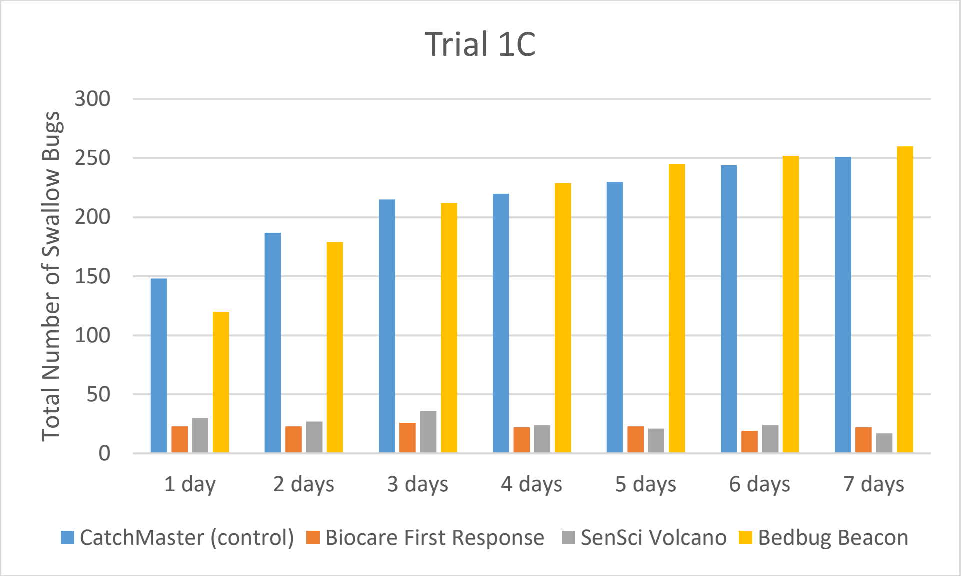

Figure 2.6. Numbers of swallow bugs collected by different traps placed in arena trial settings. Traps included a sticky card trap with no attractant that served as a control (CatchMaster 288i), a carbon dioxide based trap with a collection cup (Bedbug Beacon^{TM}), a carbon dioxide and heat trap with a bed bug pheromone on a sticky card (Biocare First Response Bed Bug Monitor), and a bed bug pheromone lure trap with a collecting cup (SenSci Volcano^{TM}). Trial 1C involved use of the same traps but did not include attractants (carbon dioxide, heat, and pheromone) as in trial 1A and 1B. A total of 1200 swallow bugs (200 per replication) were introduced into the arena setting in each trial and the figures indicate the total number captured.

A chi-squared test for contingency tables at 24 hours indicated (x^2 test stat=19.543, df=3, p-value=0.0002), indicating significance (alpha=0.05) between the traps with attractants and traps without attractants. A follow-up chi-square test was run comparing proportions of choosing control “trap” for traps with attractants versus without attractants (x^2 test stat=15.826, df=1, and a p-value <0.001), indicating that the significance (alpha=0.05) was between the control trap.

A chi-squared test for contingency tables at seven days indicated (x^2 test stat=69.941, df=3, and a p-value <0.001), indicating significance (alpha=0.05) between the traps with attractants and traps without attractants. A follow-up chi-square test was run comparing proportions of choosing control “trap” for traps with attractants versus without attractants (x^2 test stat=19.018, df=1, and a p-value <0.001), indicating that the significance (alpha=0.05) was between the control trap.

DISCUSSION

In the initial study, the BedBug Beacon trap with a carbon dioxide trap attractant had the highest trap capture at both 24 hours and after seven days, attracting 12.6-13.0% and 20.3-25.6% of the swallow bugs in the test arena. However, when the results were repeated with the same trap without the carbon dioxide attractant these traps captured nearly the same percentage after seven days, 10.0% after one day and 21.7% after one week. This suggests that carbon dioxide may not be as effective an attractant for swallow bugs as it is for bed bugs, although birds certainly ventilate carbon dioxide (Calder &Schmidt-Nielsen 1968).

A simple sticky passive trap (Catchmaster 288i) served as the control in the direct comparisons of trap efficacy. This trap captured 8.2-12.7% of the 200 bugs released after 24 hours and 17.0-21.2% after one week. When the Catchmaster 288i control trap was compared to other traps without an attractant, this trap attracted 12.3% of the 200 bugs released in the arenas after 24 hours and 20.9% after seven days. This high number of swallow bugs caught by the

Catchmaster 288i sticky trap without any attractant suggests that swallow bugs randomly wander in attempt to find a harborage location. Furthermore, the adhesive used in this trap appears to be effective in capturing swallow bugs that do contact the trap.

The BioCare First Response trap (combination of heat, pheromone and carbon dioxide trap) caught far fewer bugs in comparison to the BedBug Beacon and control traps, only 3.3-4.3% of the 200 released bugs at 24 hours and 5.8-6.5% after seven days. When attractants were not included, slightly lower proportions of swallow bugs were captured (1.8%) after both 24 hours and after seven days. This is likely due to the glue (adhesive) on the pad used in this trap. It was observed that the swallow bugs would get their front legs stuck on the pad, but would usually be able to free themselves after a few hours from the adhesive. Additionally, on a few occasions, the number of swallow bugs trapped decreased over time due to the swallow bugs escaping the adhesive of the trap.

The SenSci Volcano pheromone trap consistently attracted only a small percentage of swallow bugs and was apparently ineffective in persistently trapping bugs in the collection cup of the trap. For example, in the 1C trial when the attractants were removed from the traps only 2.5% of the 200 released bugs were trapped after 24 hours and only 1.4% were present in the trap after seven days. The Volcano trap allowed nymphs to escape from the bottom of the trap where the plastic lid clips to the “volcano” style apparatus.

Overall the chi-squared test supported a significant difference in trapping success comparing the traps with and without attractants. However, the traps that had the largest differences in trapping success with attractants and those without attractants were the same traps with design problems (BioCare First Response, SenSci Volcano) both capturing the lowest percentage of total swallow bugs released. They were also the only two traps using a pheromone; the Biocare First Response trap also used a heat source. It may be that swallow bugs were searching for a harborage location rather than being attracted by a specific attractant.

To rule out the possibility of swallow bugs being attracted to the attractants of the traps, the experiment was repeated a fourth time with a modification of design providing the swallow bugs a harborage, before being placed in the arena. The result of this trial clearly showed that a majority of swallow bugs never left the harborage location and all failed to be captured by any of the traps. From these results it appears that the tested attractants were ineffective in attracting the swallow bugs from their harborage site.

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CHAPTER III. SWALLOW BUG CHOICE TESTS

INTRODUCTION

The mud nests of the cliff swallow (*Petrochelidon pyrrhonota* (Vieillot)) provide harborage and abundant food supply for swallow bugs (*Oeciacus vicarius* Horvath). Swallow bugs also can become a problem for humans when the avian hosts vacate the nests leaving behind their ectoparasites which may then enter buildings and bite humans.

Numerous monitoring devices have been developed to detect the related cimicid that is far better known as a human parasite, the bed bug (*Cimex lectularius* L.). Most of these that are marketed use lures that include carbon dioxide, heat, pheromones or some combination of these attractants (Wang et al. 2011). Weeks et al. (2011) tested potential kairomones for bed bugs and this study indicated these can have an added effect when combined with heat and carbon dioxide. Development of a monitoring device that attracts bed bugs through a semiochemical (pheromone) could make a substantial impact on the pest management of bed bugs in the United States (Weeks et al. 2013). Much effort has been made to determine the host specific cues like carbon dioxide, octanol, and lactic acid that attract bed bugs (Wang et al., 2011; Singh et al., 2012; Aak et al., 2014), or other components derived from scent glands or fecal excrement (Siljander et al., 2008; Olson et al., 2009; Weeks et al., 2013; Ulrich et al., 2016). The olfactory systems of bed bugs have been tested and human odor has been determined to have weak influences of bed bug behavior (Harraca et al. 2012).

This approach may also be useful in attracting and controlling swallow bugs in occupied homes. The experiments conducted for this study were designed to identify attractants for swallow bugs and to test different modes of carbon dioxide production, similar to Singh et al. (2013).

MATERIALS AND METHODS

Previous observations (Chapter 2) indicate that swallow bugs scatter when placed in a new environment, both with and without lights, and immediately search for harborage sites. This experiment will test different modes of carbon dioxide, a common attractant for blood feeding insects (Barrozo & Lazzari 2004), the pheromone associated with the SenSci Volcano trap (mimics chemicals on the surface of human skin), the pheromone associated with the Biocare First Response trap (sex pheromone), heat, water, other swallow bugs, mud from the nest, and fecal matter ("swallow bug essence"), which may contain an aggregation pheromone (Mendki et al. 2014).

For these studies a simple design was used to test if swallow bugs use a chemical attractant to find a host for a blood meal. The choice test bioassay was modified from a similar approach used by Hibbard et al. (1994) and Bernklau (2016). A series of laboratory choice tests were conducted to determine if various treatments, carbon dioxide, heat, swallow bug "essence", water, pheromones were attractive to swallow bugs in a controlled environment.

The basic design of the choice test involved a 10cm diameter plastic petri dish that into which was cut two small dime sized holes in opposite ends (Fig. 3.1). Beneath each of the two holes was placed a shell vial. Each petri dish had two shell vials at opposite ends. One shell vial was used to hold the test attractant, whereas the other shell vial was used as the control.

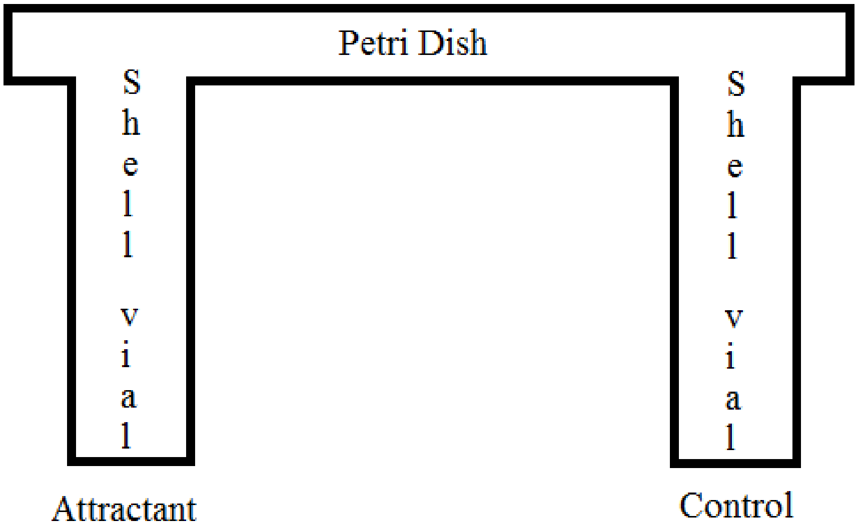

Figure 3.1. Design of the choice test bioassay which includes a petri dish that sits on top of two shell vials; one being the attractant and the other being the control side. The swallow bugs can chose either vial via a small hole cut above each of the two shell vials.

Seven attractants were tested in this trial (Table 3.1). To test carbon dioxide 1 ml of Canadian Dry^{TM} Club Soda was put in the test vial. The bed bug pheromone incorporated into the SenSci Volcano trap, a pheromone impregnated towelette (SenSci Activ^{TM}), was used in a second treatment. The third treatment was the pheromone associated with the Biocare First Response Bed Bug Trap, applied to a cotton ball placed at the bottom of the shell vial. Ten live swallow bugs (mixture of adults and nymphs) placed in the vial served as the fourth treatment. The fifth treatment used heat by placing a heat pad from the Biocare First Response trap around one of the shell vials and tied off with a pipe cleaner. The sixth treatment included dry ice which filled half of the attractant shell vial. The seventh treatment was 1 ml of tap water to rule out water as an attractant (without carbon dioxide).

Table 3.1. List of attractants used in choice test bioassay for swallow bugs (*Oeciacus vicarius* Horvath).

Attractants Used In Experiment
One ml of Canadian Dry Club Soda (carbon dioxide)
SenSci Volcano Activ Pheromone Towelette
Biocare First Response Bed Bug Pheromone
Ten live swallow bugs "Swallow Bug Essence"
Heat Pack from Biocare First Response trap design
Dry Ice
One ml of tap water

Experiments involved ten swallow bugs (mixture of adults and nymphs) placed on the center of the petri dish and covered. The choice test bioassay were kept indoors, exposed to ambient light, no artificial light and temperature was 20-21^0 C for the 24 hour course of the study. Each tested attractant was replicated five times, involving a total of 50 swallow bugs for each choice test bioassay. After 24 hours swallow bugs were recorded as being found in the vial containing the test attractant, being found in the vial of the untreated check, or as having made "no choice" by not being in either vial.

The experiment was repeated a second time, changing the design by incorporating a harborage area for the swallow bugs. This involved placing the ten swallow bugs in a small plastic cup for 24 hours along with a 2.5cm x 2.5cm piece of cardboard. The test insects settled on or in the cardboard harborage and this was then placed in the center of the choice test bioassay dish to start a trial run. The location of the insects was then recorded after 24 hours in the manner described above (Figure 3.2).

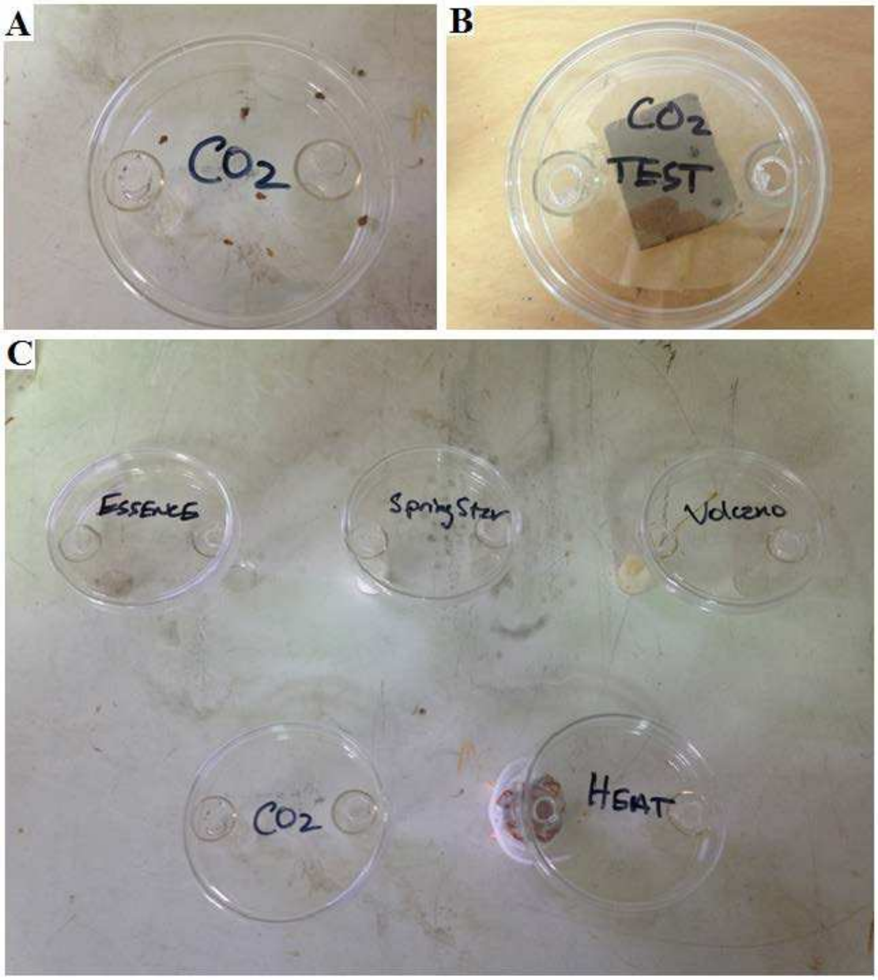

Figure 2.2. Choice test bioassay for swallow bugs (*Oeciacus vicarius* Horvath). A. The carbon dioxide test with one ml of Canadian Dry Club Soda in the shell vial on the left and the control vial on the right. B. The same test with carbon dioxide from the Canadian Dry Club Soda but this time with a harborage location for the swallow bugs, which is the small piece of cardboard. C. The setup of a few of the choice test experiments before the swallow bugs were added.

PILOT STUDY

To test the presence or absence of attractants for swallow bugs, an arena was constructed using a plastic child swimming pool by placing fine sand in the bottom to level out the pool. The circular pool had a diameter of 1.22m. Within the pool nine "climb-up traps" were deployed in a circle equally around the center of the pool. "Climb-up traps" are an easy to use monitoring plastic cup designed for bed bugs that is coated with a thin layer of talc powder which prevents bugs from crawling out. Each “climb-up trap” had a different attractant including, carbon dioxide (Canadian Dry Club Soda), carbon dioxide (sugar water and yeast mixture), carbon dioxide (dry ice), water, heat pack, SenSci Volcano pheromone, Biocare First Response pheromone, “swallow bug essence” (live mixture of swallow bug nymphs and adults) and a control. All nine traps were spread equally apart in a circle, each one about 30cm from the center of the pool.

In this experiment, 50 swallow bugs (adults and nymph mix) were removed from vacated cliff swallow nests through the use of a Berlese funnel (Neethirajan et al. 2007) and then an aspirator was used to separate 50 swallow bugs. The swallow bugs were separated and placed into a Ziploc bag with an old cliff swallow nest and allowed to acclimate for 24 hours on or in the nest and stored at 18.3°C.

After 24 hours, all of the swallow bugs found harborage in the previously removed nest in the Ziploc bag. The nest was placed into the arena with the nine “climb-up traps” and left for 24 hours. After 24 hours, no swallow bugs were captured in any of the “climb-up traps” and the experiment was discontinued (Fig. 3.3).

Figure 3.3. The pilot study experiment where trapping success of eight attractants and one control was tested using "climb up traps". From the top clockwise dry ice (carbon dioxide), "swallow bug essence", heat, sugar water and yeast (carbon dioxide), water, SenSci Volcano pheromone towelette, Canadian Dry Club Soda (carbon dioxide), Biocare First Response pheromone, and control. After 24 hours, no swallow bugs (*Oeciacus vicarius* Horvath) were attracted to the individual choices and the experiment was discontinued.

RESULTS

CHOICE TEST WITHOUT HARBORAGE

In the experiment where seven different attractants were tested without a harborage (cardboard) (Fig. 3.4), many of swallow bugs failed to be attracted after 24 hours to any of the different attractants. Overall, ninety of the 350 released swallow bugs (26%) failed to make a choice. The "swallow bug essence" attracted in 13 (26%) of 50 swallow bugs in comparison to 14 (28%) found in the control vial. The remaining 23 (46%) of 50 swallow bugs made no choice. The SenSci Volcano pheromone attracted 10 (20%) of 50 swallow bugs. This compared to 25 (50%) swallow bugs for the control, whereas 15 (30%) made no choice. The Canadian Dry

Club Soda (carbon dioxide) attracted 20 (40%) of 50 swallow bugs in comparison to 19 (38%) for the control; whereas 11 (22%) of 50 swallow bugs made no choice. The heat pack attracted 8 (16%) of 50 swallow bugs in comparison to 26 (52%) for the control; 16 (32%) of 50 swallow bugs made no choice. The Biocare First Response pheromone used in the trap attracted 13 (26%) of 50 swallow bugs in comparison to 22 (44%) for the control; 15 (30%) of 50 swallow bugs made no choice. The dry ice (carbon dioxide) attracted 16 (32%) of 50 swallow bugs in comparison to 26 (52%) for the control; 8 (16%) of 50 swallow bugs made no choice. The water choice attracted 29 (58%) of 50 swallow bugs in comparison to 19 (38%) of 50 swallow bugs for the control. In this trial only two (4%) of 50 swallow bugs made no choice.

A chi-squared test for contingency tables indicated (x^2 test stat=45.774, df=8, p-value <0.0001), indicating a significant difference (alpha=0.05) between the attractants, control and no choice group. In order to get pairwise comparisons for the five attractants a logistic regression

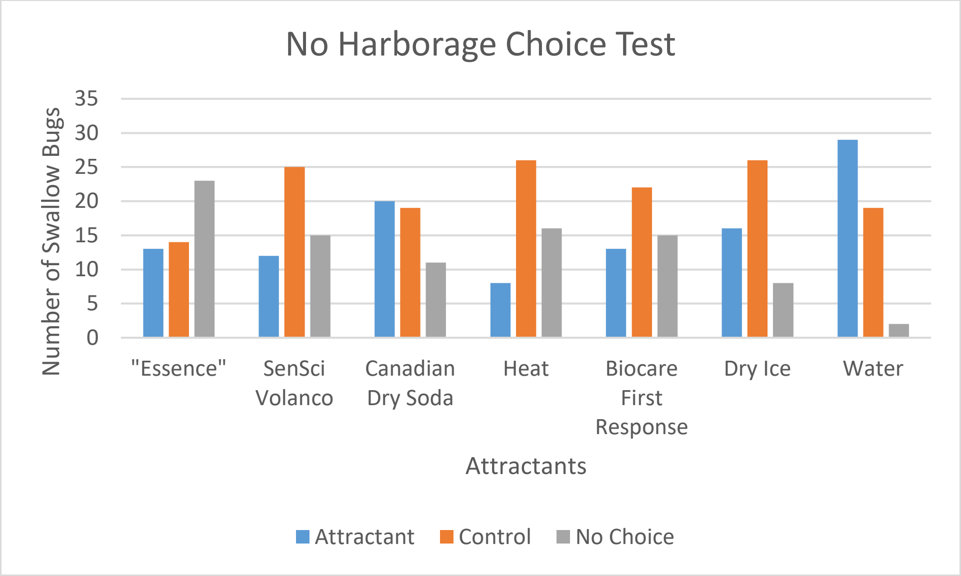

Figure 3.4. Distribution of swallow bugs in a bioassay choice tests arena of seven different attractants: swallow bug essence (live swallow bugs), SenSci Volcano pheromone, and one ml of Canadian Dry Soda (carbonated carbon dioxide), Heat, Biocare First Response pheromone, Dry Ice (carbon dioxide) and water. Each experiment included 50 swallow bugs introduced into a petri-dish containing either one of the attractants on one side at the bottom of a shell vial and an untreated control on the opposite side. Data shown indicates the number (out of 50) that either were found within the vial containing the attractant, the vial of the untreated check, or did not choose either.

was run. The response variable was choice (attractant versus other) and the predictor variable was attractant (heat vs. carbon dioxide p=0.0008, SenSci Volcano vs. carbon dioxide p<0.0001, SenSci Volcano vs. dry ice p<0.0001, SenSci Volcano vs. essence p<0.0001, SenSci Volcano vs. heat p<0.0001, SenSci Volcano vs. Biocare First Response p<0.0001, water vs. dry ice p=0.0086, water vs. essence p=0.0011, water vs. heat p<0.0001, water vs. Biocare First Response p=0.0011, and water vs. SenSci Volcano p=0.0019).

CHOICE TEST WITH HARBORAGE

In the experiment with harborage (cardboard) (Fig. 3.5) far more swallow bugs failed to make a choice after 24 hours. In this test, 249 (71%) of the 350 swallow bugs failed to make a choice, instead remaining within the harborage. The "swallow bug essence" attracted 12 (24%) of 50 swallow bugs in comparison to nine (18%) in the control. The remaining 29 (58%) swallow bugs failed to make a choice. The SenSci Volcano pheromone attracted only a single swallow bug (2%) of 50 swallow bugs in comparison to 14 (28%) of 50 swallow bugs in the control, whereas 35 or 70% of 50 swallow bugs failed to make a choice. The Canadian Dry Club Soda (carbon dioxide) attracted 4 (8%) of 50 swallow bugs in comparison to 3 (6%) in the control; 43 (86%) failed to make a choice. The heat pack attracted 9 ((18%) of 50 swallow bugs in comparison to 3 (6%) in the control; 38 (76%) failed to make a choice. The Biocare First Response pheromone attracted 6 (12%) of 50 swallow bugs in comparison to 6 (12%) in the control; 38 (76%) failed to make a choice. The dry ice (carbon dioxide) attracted 3 (6%) of 50 swallow bugs in comparison to 2 (4%) in the control; 45 (90%) failed to make a choice. The water attracted 8 (16%) of 50 swallow bugs in comparison to 21 (42%) in the control; 21 (42%) of 50 swallow bugs failed to make a choice.

A chi-squared test for contingency tables indicated (x^2 test stat=61.369, df=12, p-value <0.0001), indicating a significant difference (alpha=0.05) between the attractants, control and no choice group. A follow-up pairwise comparison using logistic regression was run to locate specifically where the significance (alpha=0.05) is located (essence vs. carbon dioxide p=0.0261, essence vs. dry ice p=0.0094, SenSci Volcano Activ pheromone vs dry ice p=0.0208, SenSci Volcano Activ pheromone vs carbon dioxide p=0.0177, SenSci Volcano Activ pheromone vs. essence p<0.0001, SenSci Volcano Activ pheromone vs. heat p=0.0003, SenSci Volcano Activ pheromone vs Biocare First Response pheromone p=0.0034, and water vs. Sensci volcano Activ pheromone p=0.0007).

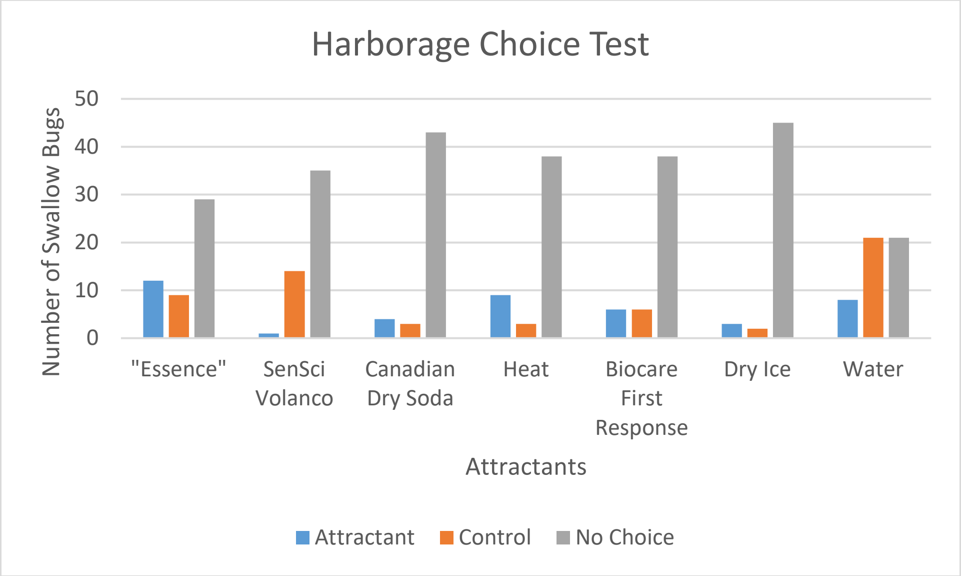

Figure 3.5. Distribution of swallow bugs in a bioassay choice tests arena of seven different attractants: swallow bug essence (live swallow bugs), SenSci Volcano pheromone, and one ml of Canadian Dry Soda (carbonated carbon dioxide), Heat, Biocare First Response pheromone, Dry Ice (carbon dioxide) and water. Each experiment included 50 swallow bugs that were introduced into a petri-dish once inside a harborage (small piece of cardboard) containing either one of the attractants on one side at the bottom of a shell vial and an untreated control on the opposite side. Data shown indicates the number (out of 50) that either were found within the vial containing the attractant, the vial of the untreated check, or did not choose either.

DISCUSSION

In both the harborage and no harborage choice tests only a small percentage of swallow bugs were attracted to the offered choices. During the first trial run where the swallow bugs were placed into petri dishes, swallow bugs would scatter and randomly fell into the shell vials which acted as a pitfall trap. After making these observations and from previous experiments, the next approach was to test if the swallow bugs required a harborage. The swallow bugs readily used small pieces of corrugated cardboard as temporary harborage; most, but not all, of the swallow bugs used the small holes of the cardboard.

Once the swallow bugs were in the cardboard pieces, they were introduced in to the petri dishes. However, in these experiments swallow bugs were not attracted to any of the attractants tested. Either the “swallow bug essence”, SenSci Volcano pheromone, club soda (carbon dioxide), heat, Biocare First Response pheromone, dry ice (carbon dioxide) or water attracted swallow bugs in numbers significantly different from the check.

Bed bugs are known to be attracted to carbon dioxide (Aak et al. 2014) but apparently swallow bugs did not respond similarly. My experimental design tested two carbon dioxide attractants with the carbonated club soda and the dry ice. Bed bugs responded most positively to the carbon dioxide produced by dry ice, except for exposure to a human host (Legrand et al. 2016). Wang et al. (2011) noted that the more elaborate trapping designs (CDC3000 and Nightwatch) were inferior in comparison to dry ice in “climb-up traps”. In these experiments, tests without harborages, few of the swallow bugs died within the 24 hour period and were tabulated as not making a choice. During the testing with a harborage, majority of the swallow bugs that made no choice were still alive within the cardboard, but still a few swallow bugs died within that time period. There were statistical differences with respect to the attractants, control and no choice groups, but there was no visible evidence that any of the attractants actually attracted in any of the swallow bugs.

It is was observed in these trials that the swallow bugs do not live long once separated from the cliff swallow mud nest. When the bugs are placed into any kind of plastic holding container or petri dish, they died within a few days unless they located a harborage on a piece of the mud nest provided.

In review, these trials did not indicate attractiveness of swallow bugs to any of the attractants offered. Several attractants that are known to attract bed bugs (e.g., heat, carbon dioxide, certain pheromones and kairomones), but these attractants failed to consistently attract high numbers of swallow bugs in my experiment. This may reflect the differences in life history of these two insects. Most of the time spent by swallow bugs occurs in the mud nest of a cliff swallow host limiting the need for long distance attractants when seeking hosts. In contrast bed bugs often establish where a host is typically present, and may feed regularly on this host throughout the year, but require some host seeking behavior as they may disperse considerable distances to harborage sites after a blood meal.

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CHAPTER IV. INSECTICIDE EVALUATIONS

INTRODUCTION

The bed bug (*Cimex lectularius* L.) has been associated with humans since the beginning of recorded human history (Usinger 1966). In recent decades, bed bugs have re-emerged as a major pest in North America due to many factors including the increasing movement of people that can carry the insects and the absence of effective insecticides, resulting from registration losses and insecticide resistance development (Krueger 2000). Since then pest control operators (PCO's) have struggled to find the next best solution to control bed bugs (Potter 2005).

There are many different types of insecticides on the market including oil-based and conventional. Control with these products can be challenging with many different factors determining success. Differences in the field and the laboratory show differences when bed bugs have the availability of a blood meal after any insecticidal exposure affecting the mortality. Protocols for insecticide efficacy should include offering a blood meal to treated bed bugs within 1 to 3 days after treatment (Singh et al. 2016). Some recent trials evaluating insecticides for bed bugs include those of Singh et al. (2014) and Moore et al. (2006).

Many pyrethroid insecticides have been developed for control of bed bugs that have shown signs of success but there have also been populations of bed bugs that have developed resistance to these insecticides. One of the first pyrethroid insecticides to combat bed bugs was deltamethrin. Seong et al. (2010) reported that two mutations in the voltage-sensitive sodium channel alpha-subunit gene of bed bugs conveyed resistance to deltamethrin. Yoon et al. (2008) demonstrated common resistance of bed bug populations to deltamethrin. Resistance to deltamethrin and cyhalotrin were detected in human dwellings in Kentucky and Ohio (Romero et al. 2007). Studies supports the pyrethroid resistance observed in *C. lectularius* from evidence that metabolic detoxification in the form of both hydrolytic esterases and microsomal oxidases (Dang et al. 2015). Adelman (2011) showed bed bug resistance to the insecticide deltamethrin (Suspend SC and Suspend Polyzone).

There has been extensive research dedicated to bed bugs and effective insecticides, but there have not been any studies available for swallow bugs (*Oeciacus vicarius* Horvath) nor indicating what insecticides are useful in controlling this insect. The swallow bug is a relatively uncommon pest (with respect to pest control) in the southern Rocky Mountain region that occurs when cliff swallows (*Petrochelidon pyrrhonota* (Vieillot)) establish nests on buildings. Swallow bug infestations of occupied dwellings occur when cliff swallows migrate in the fall and the swallow bugs remain in the nest without a blood meal. When air temperatures begin to cool at night, near the end of summer and beginning of fall, the swallow bugs frequently find their way into homes through windows, vents and roof access points or end up inside to evade predation due to destruction of the cliff swallow nest. Control efforts are complicated by these birds being protected under provisions of the Migratory Bird Treaty Act, which prevents disturbance of nesting cliff swallows until the fall when there are no birds or eggs present within the nest.

Once inside the home the swallow bugs can become a pest because of the bite habits. These experiments focused on finding products that are effective for the control of swallow bugs, evaluating common insecticides presently used for bed bugs and used an experimental design following Hirsch et al. (2016).

MATERIALS AND METHODS

An experimental design was used to test five different insecticides against swallow bugs, presently available for bed bugs control. These insecticides included: Suspend Polyzone (deltamethrin), Talstar Professional (bifenthrin), Onslaught Fastcap (esfenvalerate, prallethrin, piperonyl butoxide), Temprid (midacloprid, cyfluthrin), and Phantom (chlorfenapyr) (Fig. 4.1). The insecticides bifenthrin, deltamethrin, cyfluthrin, and prallethrin are all pyrethroid insecticides. Imidacloprid is in the neonicotinoid class and chlorfenapyr a halogenated pyrrhole. Piperonyl butoxide is not directly insecticidal but acts to synergize the effects of many insecticides, notably some pyrethroids.

Figure 4.1. Insecticides used in the experiment, testing for efficacy on swallow bugs (*Oeciacus vicarius* Horvath). A (Phantom), B (Talstar Professional), C (Temprid SC), D (Onslaught Fastcap, E (Suspend Polyzone).

Testing involved a bioassay in a 10cm diameter plastic petri dish with filter paper (90mm Cat No 1004 090 Whatman^{TM}) on the bottom. The test insecticides were first mixed with distilled water at dilutions specified on label directions: Suspend Polyzone at 0.06%, Talstar Professional at 0.06%, Onslaught Fastcap at 0.062%, Temprid at 0.75% and Phantom at 0.5% dilution rate. Each insecticide mixture of 0.5ml of the diluted mixture was applied evenly on each filter paper. This was done by mixing a desired dilution rate and then using a syringe with 0.5ml of insecticide and placing that exact volume onto the filter paper. This was equivalent to 16 drops of insecticide. The insecticide was allowed to dry ca. one hour before the test insects were introduced. A total of five different dishes were prepared for each of the five test treatments and an additional five dishes lined with filter paper served as the untreated check.

Trials were started by introducing a mixture of ten adult and nymphal swallow bugs into each dish, then covering it. Swallow bugs were collected by placing the mud nest in a Berlese funnel (Neethirajan 2007) and collecting the swallow bugs. The swallow bugs were picked up and counted with an aspirator and placed in small plastic cups. The dishes were then held in a room with ambient light from windows, but no artificial light and the air temperature were at 20-21^{0} C (Fig. 4.2).

Figure 4.2. The insecticide experiment procedure for swallow bugs (*Oeciacus vicarius* Horvath). A. The process of adding the insecticide to the petri dishes on the filter paper with the syringe. B. The insecticide absorbed by the filter paper. After about 60 seconds the filter paper was uniformly covered with the insecticide. C. Petri dishes awaiting the insecticidal treatment.

Every 24 hours the number of dead swallow bugs was recorded and observations were made daily until either all ten swallow bugs were dead or when the trial was concluded at 14 days. Determinations of whether the swallow bug(s) were alive involved using a small probe or pointed piece of wire to touch the insect and observing whether there was any movement or twitching of the legs, which was used to establish whether the insect was considered to be alive or dead (Steelman et al. 2008).

A second trial was conducted to test the residual duration of the different test insecticides. Petri dishes with filter paper were prepared in the same manner as the above trial. However, before the swallow bugs were introduced into the dishes a period of two weeks was allowed to elapse, during which time the dishes were maintained indoors at 20-21°C. A total of five petri dishes of each insecticide treatment for each residual period, plus untreated control, were included in this trial. When the trial was initiated, ten swallow bugs (mixture of adults and nymphs) were introduced into each test dish. Mortality was recorded daily until all ten swallow bugs died or until 14 days was reached. A third trial was conducted allowing a period of one month to elapse before the swallow bugs were introduced into the treated test dishes. The numbers of swallow bugs available for this final trial were more limited and only involved 30 swallow bugs per treatment, in comparison to the 50 used in the previous two trials.

RESULTS

INITIAL INSECTICIDE EXPOSURE

In the initial experiment (Figure 4.3) a majority of the insecticides caused 100% mortality at ten to thirteen days. Among treatments the order of when 100% mortality was noted was: Suspend Polyzone, 10 days; Talstar Professional, 12 days; Temprid SC and Onslaught Fastcap, 13 days. Phantom failed to cause 100% mortality at two weeks. Phantom may not reach 100% mortality due to the active ingredient chlorfenapyr, which disrupts ATP production. Since that process is slower than other modes of actions, Phantom can have a transfer effect similar to that of baits. The label claims that Phantom useful for bed bugs because of long residual and efficacy. In this experiment there was also high mortality in the control which had 29 of 50 swallow bugs die within the two week period (58% mortality).

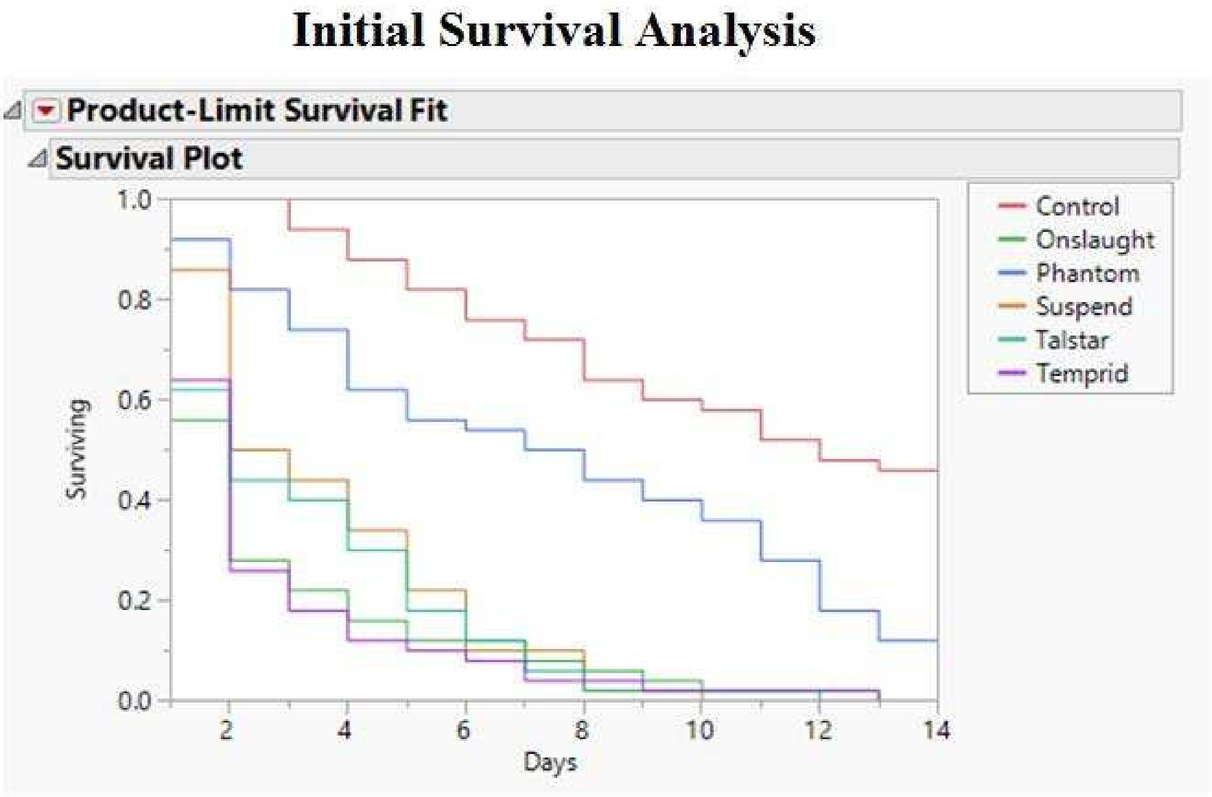

Figure 4.3. Survival of swallow bugs following exposure to five insecticides; Suspend Polyzone (deltamethrin), Talstar Professional (bifenthrin), Onslaught Fastcap (esfenvalerate prallethrin, piperonyl butoxide), Temprid (midacloprid, cyfluthrin), and Phantom (chlorfenapyr) and one control group. All insecticides were applied at the highest dilution rate allowed per the individual label directions. Each insecticidal group and control group subjected 50 swallow bugs and the survival analysis is the results of the test.

TWO WEEK INSECTICIDE EXPOSURE

On the second trial, exposing swallow bugs to insecticide residues aged two weeks, 100% mortality was achieved with Temprid SC at seven days, followed by Talstar Professional at 11 days, and both Suspend Polyzone and Onslaught Fastcap at 12 days (Figure 4.4). This trial indicated better efficacy with Phantom than in the first trial, with the 98% mortality. Mortality in the control was lower than in the initial exposure treatment but still resulted in 17 of 50 swallow bugs dying within the a two week period. It was not expected that the two week residual tests would reach mortality faster than the initial treatment tests where the insecticide would be at its highest potency.

Two Week Survival Analysis

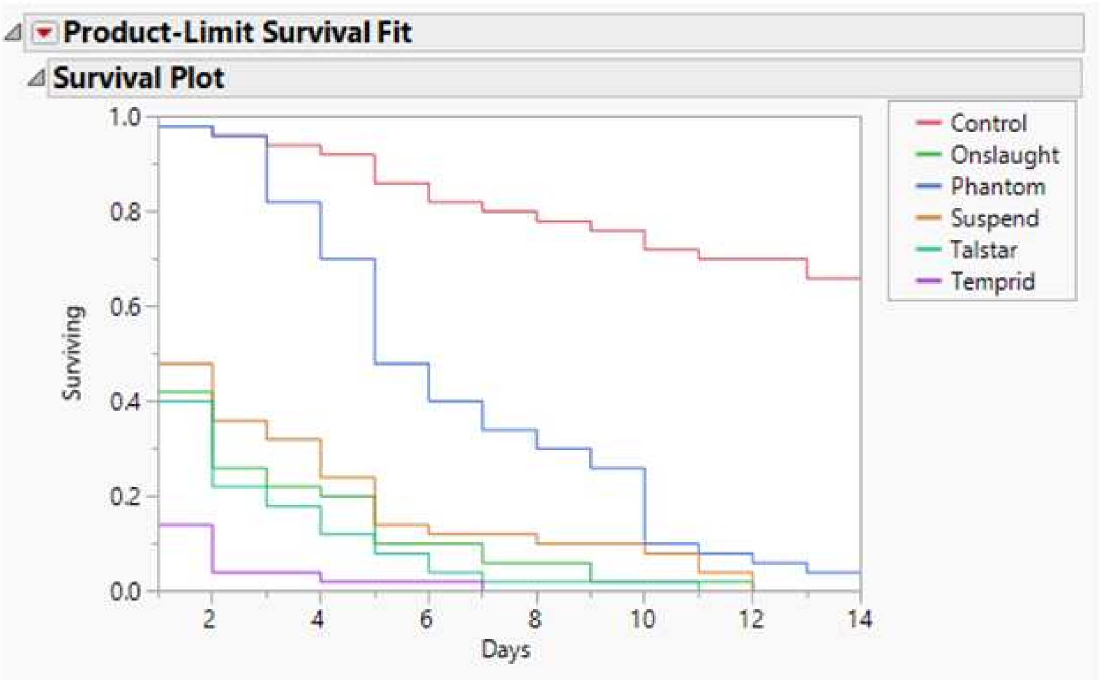

Figure 4.4. Survival of swallow bugs following exposure to five insecticides; Suspend Polyzone (deltamethrin), Talstar Professional (bifenthrin), Onslaught Fastcap (esfenvalerate prallethrin, piperonyl butoxide), Temprid (midacloprid, cyfluthrin), and Phantom (chlorfenapyr) and one control group. Each insecticidal group and control group subjected 50 swallow bugs and the survival analysis is the results of the test. All insecticides were applied at the highest dilution rate per the label directions. In this trial two weeks were allowed to pass between preparation of the treated dishes and the introduction of the swallow bugs.

In the experiment testing insecticides that had aged one month before introducing swallow bugs (Figure 4.5), it took only 6 days for there to be 100% of the swallow bugs exposed Temprid. One hundred percent mortality was achieved in 9 days following exposure to residues of Talstar Professional and 10 days with Suspend Polyzone. Onslaught approached was almost 100% mortality, with only one swallow bug surviving after one month. Mortality of swallow bugs after a one month exposure to Phantom was short of 100% mortality by two swallow bugs. In this trial the control group showed about 50% mortality over the two weeks of testing.

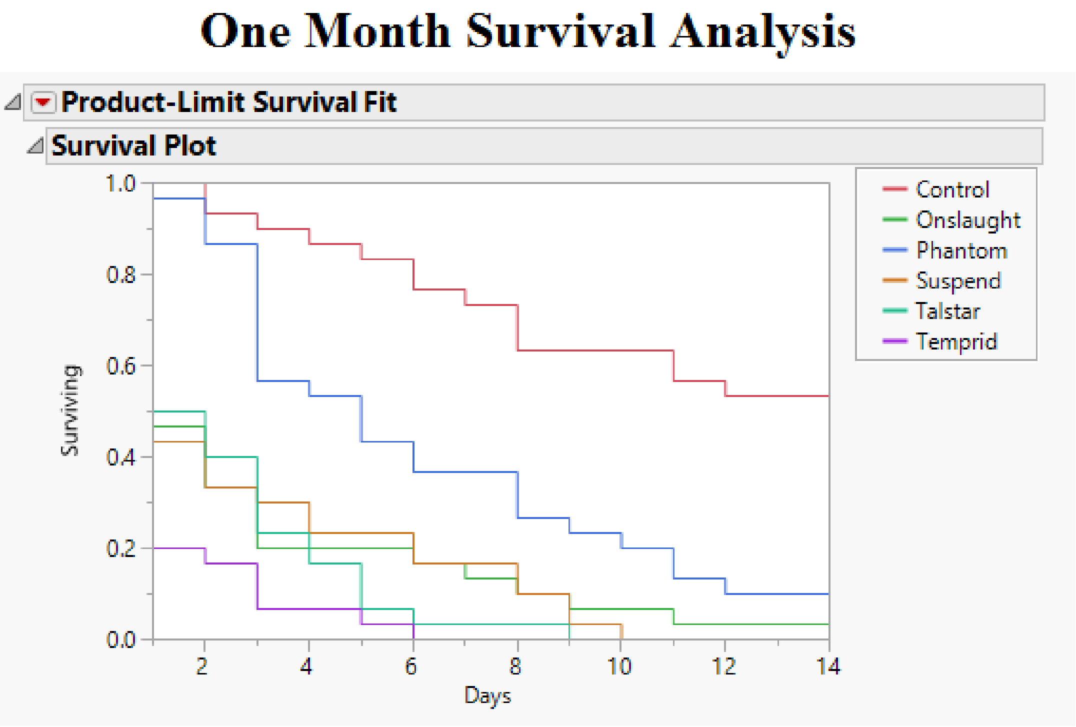

Figure 4.5. Survival of swallow bugs following exposure to five insecticides; Suspend Polyzone (deltamethrin), Talstar Professional (bifenthrin), Onslaught Fastcap (esfenvalerate prallethrin, piperonyl butoxide), Temprid (midacloprid, cyfluthrin), and Phantom (chlorfenapyr) and one control group. Each insecticidal group and control group subjected 50 swallow bugs and the survival analysis is the results of the test. All insecticides were applied at the highest dilution rate per the label directions. In this trial one month was allowed to pass between preparation of the treated dishes and the introduction of the swallow bugs.

DISCUSSION

An important consideration when using a chemical control in a home or office setting is what insecticide will be most effective. A client with a pest control problem (i.e. swallow bugs) is interested in a product that will be effective and that will give results rapidly. Unfortunately, as with bed bugs, clients have little patience concerning swallow bug control.

These trials indicated that most insecticides presently used for bed bugs are also effective against swallow bugs. Most insecticides were capable of achieving 100% mortality within two weeks, and in one case in as little as 6 days. Furthermore, aging of the insecticides for two weeks and one month before exposing swallow bugs did not result in reduced activity, and may have even produced more rapid mortality.

The one treatment that did not provide complete mortality was Phantom (chlorfenapyr). This is an insecticide that is most often used for bed bug control where resistance has developed to pyrethroid insecticides (e.g., Talstar Professional, Suspend Polyzone, and Onslaught). Chlorfenapyr has a mode of action that is very different from the other tested insecticides, all of which work on aspects of the insect nervous system, and instead disrupts ATP production. It is slower acting and higher mortality with Phantom may have been observed had this study been extended beyond two weeks.

One problem throughout these trials was very high mortality in the control group, averaging close to 50% mortality without exposure to insecticides. Observations made indicate that the filter paper used in these trials was not responsible for the high background mortality. It is suggested that the experimental design used in these trials may have resulted in excessive desiccation. It has been observed on a few occasions that the swallow bugs appear vulnerable when they are off of their mud nest, but they can be resilient and live for years in the mud nest itself.

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