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Management of Bed Bugs Using Desiccant Dust and CO_2 Activation, a Laboratory Study and a Field Trial

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Abstract

This study takes a closer look at the effect of desiccant dusts on bed bug mortality and behaviour. The effect of CO_2 as a kairomon to improve desiccant dust treatments is investigated. In a small scale Petri dish experiment it was found that Syloid 244 fp (a synthetic amorphous silica gel) outperformed Myrnix (a diatomaceous earth dust) when comparing for mortality on bed bugs. This study then looked closer at the effects of Syloid 244 fp in an arena study, and arena field simulation. Finally a method was developed to use this desiccant dust effectively in a field trail. The result of the field study was eradication of bed bugs in 5 apartments where Syloid 244 fp was combined with CO_2 stimuli in only 7 days. In 6 apartments where only Syloid 244 fp was used for 7 days all treatments failed. This study demonstrates that desiccant dust in combination with CO_2 shows potential for bed bug control.

Sammendrag

I denne studien ser vi nærmere på effekten av tørkepulver på veggdyr med tanke på dødelighet og adferd. Muligheten for å utnytte CO_2 som et kairomon for å øke effekten av tørkepulver ble undersøkt. Det ble bekreftet at Syloid 244 fp (et syntetisk amorphous silica gel) var mer effektivt mot veggdyr enn Myrnix (et diatome pulver) i et småskala petriskålforsøk. Syloid 244 fp ble derfor undersøk nærmere i et arena forsøk og et simulert felt forsøk i arena. Til sist ble det utviklet en metode for å effektivt utnytte tørkepulver i felt. Resultatene fra feltforsøket viste at vi klarte å fjerne veggdyrene fra 5 rom hvor vi kombinerte tørkepulver og CO_2 i løpet av en 7 dagers behandling. I 6 rom hvor vi kun brukte tørkepulver klarte vi ikke å bli kvitt veggdyrene i løpet av en 7 dagers behandling. Denne studien viser at tørkepulver i kombinasjon med CO_2 har et potensiale i kampen mot veggdyr.

Introduction

Systematics and distribution

Bed bugs belong to the insect order Hemiptera which includes more than 85,000 species (Brusca 2002). Members of this order are characterized by the piercing-sucking mouthparts which they use to ingest fluids. The mouthparts form an articulated beak, and the mandibles and first maxillae are stylet-like, lying in a dorsally grooved labium. The wings are normally membranous, although the suborder heteroptera have a thickened section to the wing, and the pronotum is quite large (Brusca 2002). Most hemipterans feed on plant fluids, particularly sap, but some have evolved to feed on insect and animal blood. One distinct group within the hemiptera order, the family Cimicidae, includes 91 described species which all feed on blood (Robinson 2005). They are obligate hematophags and most feed on bats or birds (Usinger 1966). Some are opportunistic and may use other vertebrates as blood sources if the primary host disappears. Important factors for the success of this host switch are the nutrient value of the host blood and if the bed bug are able to exploit the new host when they associate freely (Johnson 1941). Although many species may bite man, only two species are truly associated with humans, *Cimex lectularius* L. and *Cimex hemipterus* (F.). Their biology is strikingly similar and most life history traits and behavioural aspects of their biology are overlapping. *Cimex lectularius* can be found in most parts of the world although is less abundant in tropical regions, while *C. hemipterus* is mostly found in tropical to sub-tropical areas mainly within the 30^{o} latitudes (Usinger 1966). One reason for this is that *C. lectularius* prefers temperatures that are 2-4 ^{0}C lower than what C. hemipterus prefers (Usinger 1966). *Cimex lectularius* is often denoted the 'common' bed bug, while *C. hemipterus* normally is termed the 'tropical' bed bug. However, in this manuscript, the term 'bed bug' will be used in discussions on *C. lectularius* and if *C. hemipterus* is discussed, then this will be clearly noted.

Appearance

Adult bed bugs are flat and oval (Fig. 1). Adults are dark brown coloured and approximately 5 mm in length (Usinger 1966). The proboscis of the bed bug is three-segmented and rests in a grove beneath the head and thorax, when in repose (Robinson 2005). They have a four-segmented antenna (Robinson 2005). Important morphological characteristics include the pronotum that is more than 2.5 times as wide as long at the middle, and the anterior part of the pronotum is strongly U-shaped and covered in seta (Usinger 1966). The shape of the pronotum is used to distinguish *C. lectularius* from *C. hemipterus* whereby the pronotum in the latter species is less than 2.5 times as wide as long at middle and the anterior part is weakly U-shaped with a moderate cover of seta (Usinger 1966). Eggs of the bed bugs are approximately 1 mm long, oval shaped, cream white in colour and are glued to the surface when laid. First instar

nymph are somewhat transparent and light in colour until they feed. The 2nd-5th instar nymphs increase their size 1.2 times on average and turn a darker brown with each consecutive moult (Usinger 1966). The nymphs look like smaller versions of adult bed bugs, although the former do not have wing pads (hemelytra). The tarsi of the nymphs are two segmented and adult bedbugs have three segmented tarsi.

Figure 1. *Cimex lectularius* L (ill. Hallvard Elven)

Life cycle

Males mate the female by a process known as 'traumatic insemination'. The males penetrate the female abdomen on the right side by using a hardened, needle-like intromittent organ (Siva-Jothy & Stutt 2003) and the sperm is inseminated directly into the female body cavity. Females have developed the paragenital sinus where the male penetrates the abdomen (Siva-Jothy & Stutt 2003). This organ contains haemocytes that protect the female bed bug against infections and assist in healing the wound (Siva-Jothy 2006). From here the sperm enters the female's blood and migrates into a pair of sperm-storage organs connected to the oviducts. From these organs, the sperm can move up the oviduct and fertilize the eggs (Siva-Jothy & Stutt 2003). Recently fed female bed bugs are more attractive to male bed bugs and in laboratory studies are copulated on average five times by multiple males just after feeding (Siva-Jothy 2006). The reason for this is that they are slow to resist copulation when newly fed. Unfed female bed bugs can avoid copulation by pressing the paragenital sinus toward the substrate thereby making it less accessible to the males (Siva-Jothy 2006) or by releasing alarm pheromones (Kilpinen et al. 2012). Multiple traumatic inseminations will greatly reduce the female fitness leading to a shorter lifespan and decreased reproduction rate (Siva-Jothy 2006). A fed female lays on average one egg per day (Polanco *et al*. 2011). It is estimated that bed bugs, at least in theory and under optimal conditions, can lay up to 200 to 500 eggs in a life time (Usinger 1966). Adults feed approximately once per week (Usinger 1966), but may feed more regularly if a blood meal is constantly available (Pereira et al. 2013). Bed bugs have evolved to take large blood meals

rapidly. Females ingest larger meals than male bed bugs, increasing body weight by 2.5 and 1.3 times respectively (Aak & Rukke 2013). Nymphs can increase their body weight up to 6.1 times (Usinger 1966). The nymphal stage has to feed once to be able to moult into the next stage. The length of the life cycle decreases with temperature up to an optimum of 30 ^{0}C where it takes 24.2 days to complete (Usinger 1966). Bed bugs can survive long periods without food and this is also effected by temperature. At 10, 18, 27 and 37 ^{0}C bed bugs can survive on average for 401.9, 175.6, 43.4 and 17.4 days if fed once (Usinger 1966). At low temperature (<10 ^{0}C) adult bed bugs and 5th instar nymphs can survive for a long time without a blood meal (Usinger 1966). First instar nymphs when newly hatched can live up to three months without blood (Usinger 1966).

Behaviour

When bed bugs have completely engorged, they immediately seek a harbourage to hide. They tend to stop when in contact with other bed bugs and this 'thigmotaxic' behaviour aids in finding safe harbourages, typically in cracks and crevices close to the host. Wooden frames in box-spring mattresses, behind skirtings, and cracks in the wall are favourite sites. Bed bugs prefer harbourages that contain aggregation pheromones, which they detect with the pedicel of the antenna (Olson *et al.* 2009; Siljander *et al*. 2007), and they prefer the company of conspecifics within their refuges. This aggregation behaviour protects the insect from detection by their host, increases the chance of mating and helps reduce the loss of water (Benoit *et al*. 2007). Because of this behaviour, bed bugs are concealed most of the time. They mate, moult and lay eggs in this cryptic location until they require a blood meal or are disturbed (Usinger 1966). If disturbed in their harbourage, the bed bugs will release alarm pheromones that will result in the evacuation of their refuge by other bed bugs (Benoit 2011). When starved, a bed bug locates its host in three steps. Firstly appetitive searching occurs where the bed bugs are able to detect a cue or behave in a manner that optimizes the chance of finding a cue. Secondly orientation towards the host occurs when a host cue is detected, and lastly host contacts where the bed bug finds its host and is able to feed (Reinhardt & Siva-Jothy 2007). The known host cues include heat and kairomones such as CO_2 and other body odours. The bed bugs can detect CO_2 as a host cue from at least 1.5 meter (Reinhardt & Siva-Jothy 2007; Weeks et al. 2011). The insect prefers to be active at night time (scotophase) when the host is most likely to be asleep (Romero *et al*. 2010a) and the risk of detection is minimal, but they are also activated by host cues at daytime (Aak *et al*. 2014). A host seeking bed bug shows positive thermotaxis, the movement towards an up or down gradient of temperature, but when engorged show negative thermotaxis (Reinhardt & Siva-Jothy 2007; Reis & Miller 2011). This results in the bed bugs spending as little time as possible close to their host where the risk of harm and detection is the greatest. The more starved the bed bugs are, the more active they will be until five weeks without food when the spontaneous activity will decline

(Romero *et al*. 2010a). This is an adaptation to preserve energy in the absent of a host. Starved bed bugs will show a movement like the appetitive search pattern, while starved bed bugs presented with a host cue will show a more directional search pattern towards the host (Aak *et al*. 2014; Suchy & Lewis 2011).

Human association

One theory is that bed bugs first came in contact with humans who lived in caves shared with bats in southern Europe or Middle East. From there they followed humans when they started early settlements and entered into the more advanced human civilization (Usinger 1966). The earliest records of bed bugs associated with humans are from Egyptian tombs 3550 years ago (Panagiotakopulu & Buckland 1999). Other early records of bed bugs in human dwellings are from Greece around 400 B.C, Italy in 77 A.D., and China in 600 A.D. (Usinger 1966). Bed bugs are recorded in Germany by the 11th century, in France in the 13th century and in England in the 16th century (Usinger 1966). In North America the first reports of bed bugs dates from the 16th century (Koganemaru & Miller 2013) and in Australia the first reports of bed bugs are from the 18th century (Doggett & Russell 2008). In the 19th century, there was a big rise in bed bug infestations, probably a result of houses being warmed all year around by central heating system. This rise lasted until the end of the 1930's (Usinger 1966). Then with the use of insecticides (particularly DDT) from the end of the 1940's, bed bugs were almost eradicated in many developed countries. By the end of 1990's reports of bed bug infestations started to rise again (Doggett *et al*. 2012). Over the last decade reports on bed bugs have suggested that they have been on the rise all over the world (Bencheton *et al*. 2011; Lee *et al*. 2008; Lewis *et al*. 2013; Ter Poorten & Prose 2005; Wang & Wen 2011; Williams & Willis 2012). In Norway, there has also been an increase in the number of reported bed bug treatments in recent years as reported from a survey conducted on pest management companies (FHI 2014, Fig. 2). The increase of bed bugs worldwide is believed to be largely as a result of pesticide resistance, with other contributing factors including increased passenger travel and a higher general fluidity of persons and goods connected to labour, poor pest management practices, and possibly increases in the trade of second hand furniture. Up until recently, most people do not know how to identify a bed bug or know what to do if they have an infestation, however few tolerate having a blood sucking insect living in their bed. The negative view on bed bugs poses a challenge to pest control management, as complete eradication of an infestation is the only satisfactory outcome for most people.

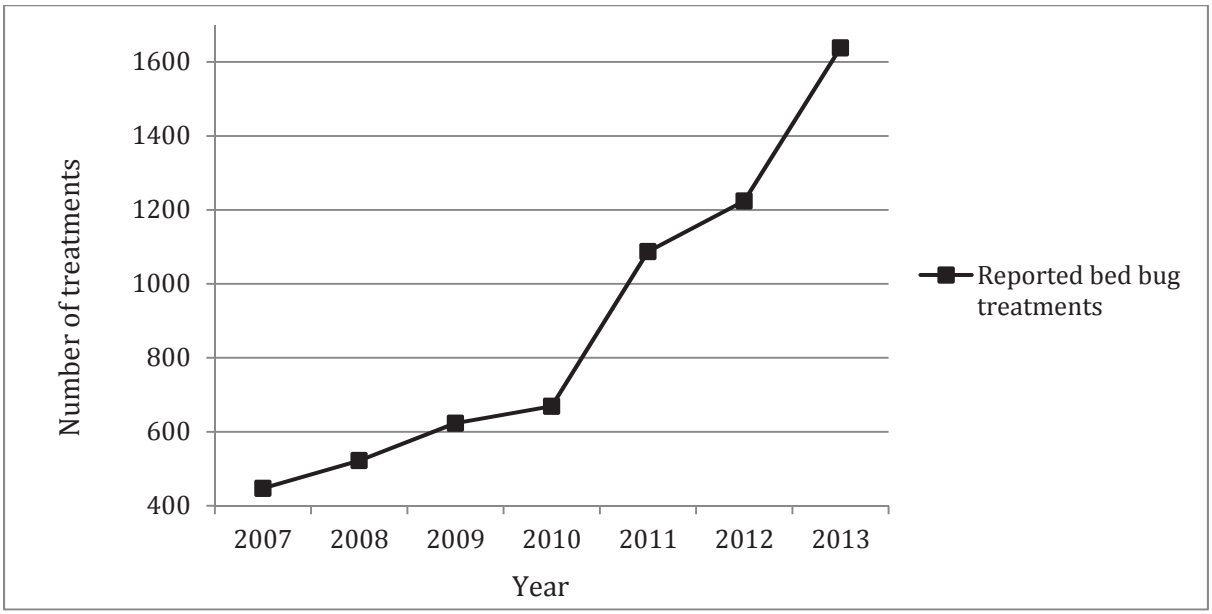


Figure 2. Number of reported bed bug treatments by pest control companies from 2007- 2013 (FHI 2014).

Bed bug management practices

Integrated pest management (IPM): IPM is the recommended method of managing with pest infestations, including bed bugs. IPM involves five steps; the first is prevention and/or early detection of an infestation. It is important to detect bed bugs at an early stage as eradication is much easier and cheaper for the client. The second step is identification of the bed bug species and confirmation of the infestation. There are other insects like cockroach nymphs and carpet beetle larvae that are easily mistaken for being bed bugs. Also some cimicidae species that live in bat or bird colonies associated with buildings would require a different approach to solve the problem. The third step is education of all involved in the eradication efforts. This should include how to avoid spreading infestations, what steps to take to help eradicate the infestations, and how to monitor after a treatment to detect re-infestations or failed treatments. The fourth step includes inspection and application of the treatments combining non-chemical means of control and insecticide application. This is to identify where the infestation is present and to ensure that the correct insecticides are applied in the most appropriate location. Non-chemical treatments are important to implement as these methods reduce the amount of insecticide required, thereby reducing non-target affects such as minimizing possible human exposure. The final step is to undertake follow-up inspections for efficacy evaluation and the possible application of additional treatments, as required (Koganemaru & Miller 2013). A Code of Practice is an industry standard based on the principle of IPM that aims to provide the best most appropriate advice of how to manage active bed bug infestations and to minimize potential bed bug infestations, both for pest managers and other stakeholders such as businesses (e.g. the hospitality industry) at risk of bed bug infestations. The first Code of Practice was developed in

Australia in 2005 (Doggett 2005) and was later adopted by Europe (Bedbugfoundation 2013), and a subsequent Code of Practice was developed in the USA (NPMA 2011). One of the main challenges with bed bug control options today is that there are numerous products claiming to be affective against bed bugs but scientific documentation from field trails verifying efficacy is lacking. Presently there is no easy, cheap and completely safe method to treat bed bug infestations. The implications of this are that bed bug management is time consuming and control is expensive, and difficult to achieve. Keeping rooms vacant is not an control option in most cases because at 22^0C blood fed bed bugs can survive on average for 135 days (Doggett *et al*. 2012). A solution to these challenges is needed.

Chemical treatment: The main insecticides used for pest control today are the pyrethroids and insect growth regulators (IGRs). In some parts of the world the carbamates and organophosphates are still employed. New chemicals that are being employed against bed bugs include the neonicotinoids and arylpyrroles (Doggett *et al*. 2012; Koganemaru & Miller 2013). Bed bugs have been demonstrated resistant to several of the most widely employed pyrethroids (Kilpinen *et al*. 2011; Moore & Miller 2009; Romero *et al*. 2010b; Tawatsin *et al*. 2011). Insect growth regulators are commonly used in combination with pyrethroids to prevent the bed bugs from completing their life cycle. IGRs are slow acting and as the insects need to feed before it can moult for the IGR to effect the moulting process before the insects die, IGRs do not protect people from being fed upon before the chemical works (Doggett *et al*. 2012). One study also showed that bed bugs are not effected by the application rate recommended with two of the most commonly used IGR products employed in the USA (Goodman *et al*. 2013). Organophosphates and carbamates are not registered for indoor usage in the USA (Koganemaru & Miller 2013). In Europe, organophosphates and carbamates are not permitted for use under the biocide directive (Biocidedirective 2014). Neonicotinoid products have shown to be effective on direct topical application but less effective via residual application (Doggett *et al*. 2012). Another study recently demonstrated that bed bugs also are resistant to two products used in the USA that contains pyrethroids and neonicotinoids (Goddard 2013).The arylpyrrole chlorfenapyr is a contact and stomach poison which when it is activated inhibits the production of ATP in the mitochondria of the insects (Koganemaru & Miller 2013). It is slow working and bed bugs are able to lay eggs before they die (Moore & Miller 2006). Studies against bed bugs have demonstrated variable efficacy with this insecticide (Doggett *et al*. 2012).

Chemical treatments come in three main formulations for in building use; liquids, dusts and aerosols. Liquid formulations are typically used along ceiling, wall junctions, base boards, cracks and crevices. Dust formulations are applied to wall outlets, wall voids and on floor under baseboards. Aerosols are commonly used to mattresses, box spring furniture and cracks and

crevices, as a direct spray at the insect rather than a space spray. However, because of the above mentioned resistance and efficiency issues, alternatives to these more traditional insecticide treatments should be employed in combination with various non-chemical treatments (Bedbugfoundation 2013). Insecticide treatments have also been associated with a potential adverse health reaction in people. Therefore chemical treatments should where possible, preferably be kept to a minimum (Centers for Disease & Prevention 2011; van Balen et al. 2012).

Desiccant dust: These products are dust formulations that results in the prevention of the insect's ability to reduce water loss thereby leading to desiccation and death. Diatomaceous earth is the fossilized remnant of hard shelled algae that are grinded to dust. Silica dust is a synthetic amorphous silica gel with a large internal surface area. Both damages the thin wax of the insects epicuticule (Ebeling 1971). Both diatomaceous earth and silica dust are candidates for the control of bed bugs due to their low toxicity to mammals and long residual effect (Ebeling & Wagner 1959). Presently, the desiccant dusts have been mainly limited to less visible application sites like wall outlets, wall voids, and on floor under baseboards because the products, especially diatomaceous earth, are obvious and unsightly (Koganemaru & Miller 2013). This may be a precaution based on practice from dust formulated chemicals. The potential of desiccant dusts has been demonstrated in some laboratory studies (Akhtar & Isman 2013; Anderson & Cowles 2012; Benoit et al. 2009b; Ebeling 1971; Wang et al. 2013) but are poorly documented in field trails.

Temperature treatment: Cold treatment of bed bugs can be undertaken by using liquid CO_2, liquid nitrogen or via freezing compartments to kill the insects. Liquid CO_2 comes in pressurized bottles and to kill the bed bugs, a direct hit at a short range is necessary to achieve the lethal temperatures (Koganemaru & Miller 2013). Using liquid CO_2 or liquid nitrogen is a time consuming procedure as the gas needs to be applied into all cracks and crevices where bed bugs are hiding. This method uses high pressure gas and can blow insects about non-lethally. When using a freezer to kill bed bugs of all stages, temperature as low as -20^0C for up to 2 days must be maintained (Benoit *et al*. 2009a; Naylor & Boase 2010; Olson *et al*. 2013). One way to use freezing effectively is to use a transport container re-built to be a freezer. For heat treatments, steamers can be employed, along with heating tent/bags, and even the heating of whole buildings. To kill a bed bug with a steamer it is necessary to contact the insect with the steam at short range to be most effective, without potentially blowing bed bugs about (Puckett *et al*. 2013). When using heating tent/bags or heating a whole building, the minimum lethal threshold required to kill all bed bugs where they are hiding is 55^0C (Kells & Goblirsch 2011; Pereira *et al*. 2009). This temperature will instantly kill all stages of bed bugs. Also important is that bed bugs

are not able to escape the heat treatment to areas that are not heated sufficiently to the lethal threshold.

Other applied methods: This includes vacuuming, bed bug proof mattress encasement, cleaning, disposal of infested furniture, reduction in possible refuges, and traps. Vacuuming if executed thoroughly is a fast and effective way to remove many bed bugs in an infestation. It also helps remove dead bed bugs, eggs and cast skins, making follow up inspections easier. Removing dust and dirt will also improve the effect of chemical treatments (Bedbugfoundation 2013). It is however important to remove and properly seal the vacuum bag after use, and preferably heat or cold treat the bag before disposal. The vacuum should be kept in a sealed container and possible decontaminated to avoid a potential risk of spreading the infestation. Mattress encasements are a fast way to treat an infested bed, which can seal an infestation inside assuming that the encasement has been constructed such that nymphs can not escape through zippers and that the encasement contains a membrane that bed bugs can not bite through. It is however important that the mattress encasement fits properly and can not readily be torn apart. Another benefit from using mattress encasements is that it conceals all old bed bug spotting from the infestation, making follow up inspections much easier and detection of re-infestation more likely (Cooper 2007). Keeping a home clean and clutter-free will remove potential hiding places for the bed bugs, making other treatments methods more efficient and increase the ease of detection of a bed bug infestation at an earlier stage. Disposal of infested furniture is a fast way of reducing a bed bug population, but using a freeze-container or a heat tent may make this an unnecessary action. However, this can be an option if the furniture is worth less than the cost for a treatment. It is important to wrap the infested item in plastic before removal, to ensure that bed bugs do not drop off the item when carted out of the premise. The items should also be rendered unusable to prevent someone from reusing the item and spreading the problem. Removing possible harbourages by caulking cracks and crevices will reduce possible hiding places, assist in early detection, and improve the effects of eradication attempts. Simple interceptor traps or climb-up traps have been shown to effectively reduce bed bug populations in combination with other treatments (Wang *et al*. 2012). Climb-up traps has also been shown to be more effective at detecting bed bug infestation than visual inspections and thus act as an efficient monitoring tool (Wang, C. *et al*. 2009). Testing traps baited with CO_2 was found to be more effective at luring bed bugs than a combination of heat and skin kairomones (Anderson et al. 2009; Wang, C. L. *et al*. 2009).

Master thesis

This Master thesis aims to investigate desiccant dusts and their effects on bed bugs. Two types of desiccants were evaluated; Syloid 244 fp (GRACE GmbH & CO., Germany) which is a synthetic

amorphous silica gel dust (Syloid) and Myrnix (Tergent AB, Helsingborg), which is a diatomaceous earth dust (DED). As mentioned above, desiccant dusts have a very different mode of action where they act physically rather than physiologically, by damaging the thin wax of the cuticule resulting in desiccation and eventual death. This makes desiccant dust an option for use against bed bugs that show resistance to pyrethroids and other insecticides. Their low mammal toxicity, long shelf-live, and long residual effect makes desiccant dust a good potential option for bed bug control, and is in accordance to IPM when used with other means of control. Though there are publications that demonstrate that desiccant dusts are effective in laboratory studies there are few studies that have measured the effect of these products in the field. This study investigates the efficacy of desiccant dust both in the laboratory and in the field situation. An important factor for the success of a desiccant dust treatment is that the bed bug comes in contact with the powder. This study will therefore examine the interaction between bed bugs and CO_2. The use of CO_2 to enhance the efficacy of bed bug treatments have, to our knowledge, not been investigated to date.

Material and Methods

Bed Bugs

The two laboratory strains used in the study were collected from different buildings in Oslo (Norway) and kept in the laboratory since 2009. The bed bugs were maintained in 140-mL polyethylene boxes stored in a climate chamber at 22 ^{0}C, 60 % RH and a 16:8 hour day: night light regimen. Daytime was from 00:00 to 16:00 and the night-time was from 16:00 to 00:00. The bed bugs were fed on human blood artificially every second week according to the feeding regimen described in (Aak & Rukke 2013). Test bed bugs were chosen from 5th instar nymphs that were fed and given two weeks to moult. Adult bed bugs were sorted into separate boxes of males and females and fed to full engorgement as described below, before they were used in the experiment.

Experiment 1 – Dose-response

The aim of this experiment was to test the differences between the two dust products, Syloid and DED, by studying mortality and abnormal behaviour caused by different application rates of the two products

Equipment: Six Petri dishes (90 mm in diameter) each per application rate were employed with the desiccant dusts and the control. Filter paper covered the bottom of the Petri dish. The

experimental setup is demonstrated in Figure 3. The temperature and humidity was recorded in the room using Tiny tag data loggers (Precision Technic Nordic, Norway). The measured average temperature during experiments for Syloid was 22 ^{0}C and relative humidity in room was 11 % RH. For DED the measured average temperature during the experiments was 24 ^{0}C and relative humidity in room was 36 % RH. Powder were weighed to 0.001g precision at a precision scale (Sartorius BP211D; Sartorius AG, Götingen, Germany) and stored in a small glass container before being transferred to Petri dishes.

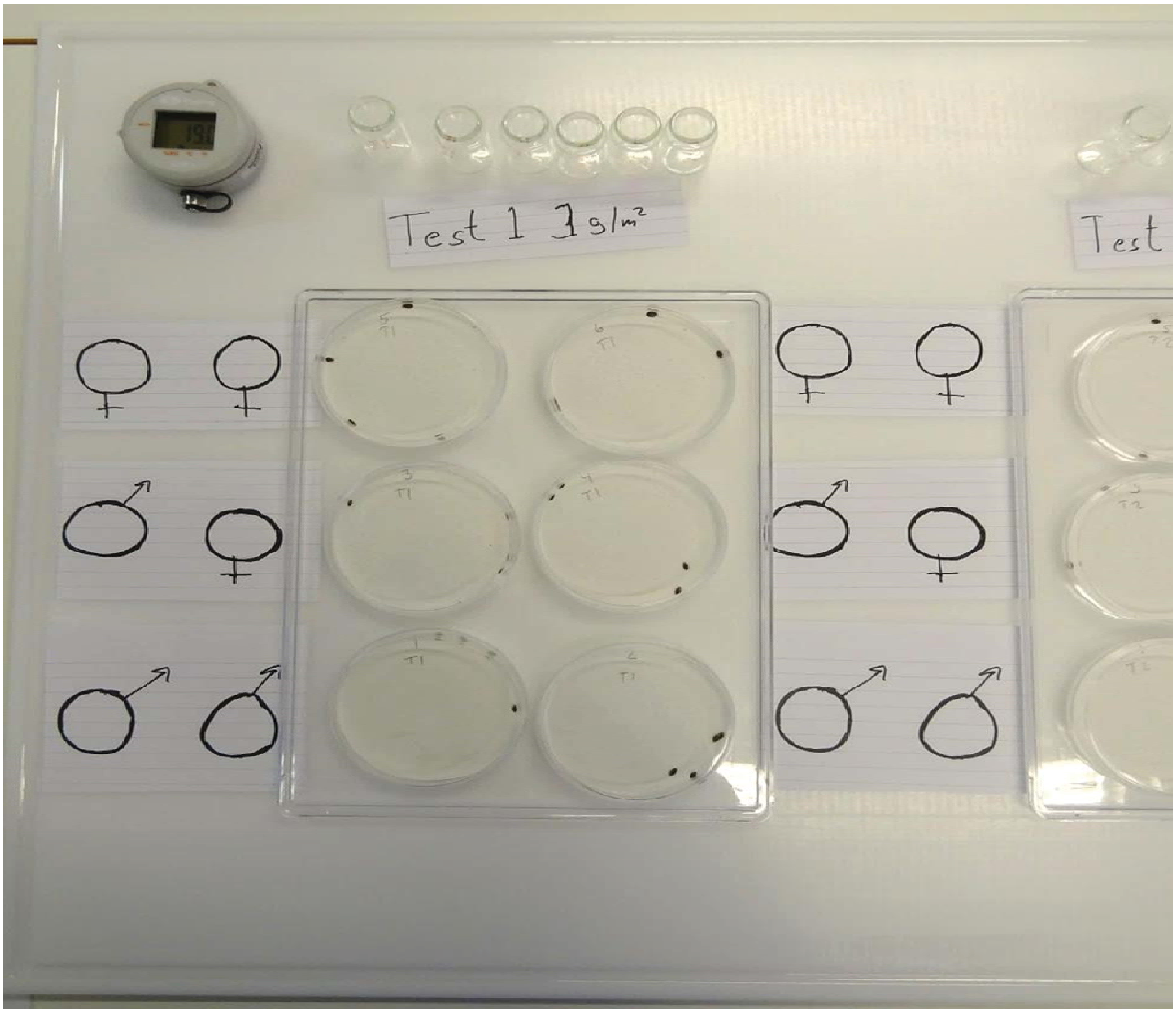


Figure 3. Dose-response setup with 6 Petri dishes containing 4 bed bugs split in gender as shown in the picture.

Experimental protocol: In total 24 adult bed bugs were employed per application rate of desiccant dust and for control (totalling 12 males and 12 females). The bed bugs were feed to repletion 24 hours before the test procedures. Four bed bugs were placed in each Petri dish. Two Petri dishes contained four male bed bugs, two Petri dishes contained two male and two female bed bugs, and two Petri dishes contained four female bed bugs per application rate of desiccant dust. The experiment was done in a laboratory where day and night regime could not be controlled and therefore followed the light regimen at the time of the experiment in Oslo,

Norway in March for Syloid and May for DED. During the experiment the time of death for individual bed bugs were recorded along with the level of aggregation in each Petri dish. If the bed bugs failed to move after gently prodding with forceps, they were considered dead. The status of bed bugs was recorded every hour for the first 12 hours, then every 12th hour for one week, and thereafter every day until all bed bugs were dead. After two weeks the experiment was terminated. For Syloid, it was recorded if the bed bugs were in an aggregation or were situated separately, every hour for the first 12 hours. For DED this data was recorded only for the 12th hour. Aggregation level was described on a category scale ranging from 1 to 5. If four bed bugs were touching each other, then this was scored as a full aggregation with the value of 5. If three bed bugs were touching and one was separate on its own the value 4 was scored. Two separate aggregations of two bed bugs each were given a score of 3 and if two bed bugs were touching and two bed bugs were separate this was scored as a 2. Four bed bugs separately positioned were scored as 1.

Desiccant dust treatment: The effect on bed bugs of the two desiccant dusts Syloid and DED were tested at application rates of 3.0, 1.0, 0.3 and 0.1 g/m^2 and compared to a control without desiccant dust (filter paper only). The dust was applied to the Petri dish, which was gently shaken to evenly distribute the powder.

Experiment 2 – Arena studies

The aim of this experiment was to test whether activation with CO_2 will increase mortality and activity of bed bugs with and without Syloid dust

Equipment: Three arenas where used that were made of plastic boards (Makrolon 150x150x4 cm). Mounted on the base board was a square frame (130x130x20 cm) with an inner area of 126x126 cm. The setup is demonstrated in Figure 4. The inside of the box had a paper sheet (128x128 cm) that provide the bed bugs with a surface that is easy for them to walk on. The arena contained four harbourages in the corners of the arena for the bed bugs to hide in. The hiding places were made of dark red Plexiglas (10x10x0.2 cm). Two 0.2 cm high pieces of plastic were placed under the harbourages corner which pointed toward the middle of the arena, so the bed bugs could crawl under it. In the centre of the arena an inverted Petri dish (5.5 cm) was placed. This was used underneath a Petri dish that contained the dry ice (solid carbon dioxide). Above each arena, light was provided by use of eight LED lists consisting of four LED strip lights (art. no 36-3848 Model XH-B01020, Clas Ohlson, Norway) put inside a plastic tube. Four of these had red film to provide night light. The other four provided the day light. Day light tubes were connected to a timer (ESIC 32-8618 AX 300, Clas Ohlson, Norway) while the red night light was kept on continually. The same light regimen was used as in the climate cambers where the bed

bugs were kept. For videoing, a web camera was employed (LIVE! CAM Socialize HD 1080, creative labs (Irl) Ltd., Ireland). The camera was mounted over the arena and connected to a computer. Every arena had a climate logger (Tinytag, Precision Technic Nordic, Norway) for recording temperature and humidity. The temperature in the room was kept stable by an air-conditioner set at 22 ^{0}C and a 10 L bucket filled with water was placed in the room to increase humidity. The measured average temperature during experiments was 22 ^{0}C and relative humidity in room was 14 % RH. Syloid was used as the desiccant dust treatment at an application rate of 0.3 g/m^2. Dry ice was used as the CO_2 source to initiate movement in the bed bugs. The average evaporation of the dry ice (±SE) for this experiment was 0.36±0.02 g/min (range: 0.15-0.45 g/min) per arena.

Figure 4. Arena studies with three arenas in the same room. Dark squares in the corner are hiding places for the bed bugs. Light and camera mounted straight above all three arenas.

Experimental protocol: Bed bugs were blood fed to repletion and then kept in the climate chambers for three days where after they were put into the arena and given another three days to acclimate. Hence, the experiments were initiated in the arenas 6 days after blood feeding. The bed bugs were collected from the arenas 10 days after feeding when the daytime videoing was completed. Surviving bed bugs were put in the climate chamber and mortality was recorded

every day up to 20 days after feeding. Syloid was distributed in the arena at 09:00 the day the experiment was begun (Day 6 after feeding). A pre-measured amount of Syloid was evenly distributed in the entire arena, except along the edges where 13 cm wide paper strip was placed. This provided the bed bugs with a 13 cm wide corridor of untreated space along the walls. Therefore, 1 m^2 in the centre of the arena was treated with the dust. A sieve was employed to evenly apply the desiccant dust. The paper strip along the edge was then removed.

In experiment without CO_2, an empty Petri dish was used and in experiments with CO_2 a Petri dish was used with dry ice as the CO_2 source. The Petri dish with CO_2 was weighed before and after use. To observe the effect of CO_2 the following procedure was employed from day 6 to day 10 after feeding: for experiments without CO_2 an empty Petri dish was placed into the arena 30 minutes after videoing began. For the experiments with CO_2, a Petri dish with dry ice was placed into the arena 30 minutes after videoing was initiated. Videoing lasted for 2 hours from 11:30 to 13:30. When the videos were analysed, the following criteria was used to score movement. Movement was recorded manually every minute for 120 minutes. If one bed bug moved a score of 1 was given. A score of 2 indicated that two different bed bugs moved and so on up to a maximum score of 10 where all bed bugs in the arena moved for that particular minute. The score was reset at the start of every minute.

Between each experiment, the arenas were cleaned by vacuuming and using dust clothes. Every part of the arena was vacuumed six times with a vacuum connected to a brush and then wiped twice using two dust clothes (JIF-tørrmopp, Lilleborg AS, Norway). This procedure was repeated twice. A separate control experiment was run with all three arenas after a cleaning to examine if there were any residual effects, however no mortality was detected in the controls up to 20 days after feeding.

Treatments: To ensure that the arena and CO_2 did not induce any mortality, the clean arenas without CO_2 and clean arenas with dry ice as a CO_2 source were evaluated. In these two tests, 10 bed bugs (5 male and 5 female) were placed in each of the arenas for total of 6 arena replicates without any killing agents present. These tests acted as a control to the Syloid treated arenas. To investigate killing efficiency of Syloid dust, arenas that contained Syloid were used. The Syloid dust treatments were either combined with an empty Petri dish or a CO_2 activation source. A total of four arenas with ten males, four arenas with ten females and four arenas with five males and five females equally divided between the two different control approaches, were used. To check for mortality bed bugs were gently prodded just prior to initiation of videoing.

Experiment 3 – Arena field simulation

The aim of this experiment was to test the effect of a heterogeneous environment on Syloid dust in combination with CO_2-activation

Equipment: The arenas were the same as in the arena studies with additional harbourages (Fig. 5) made out of four transparent plastic lids (10.5x17.5 cm) and a transparent plastic rod (130x4x1 cm). The measured average temperature during experiments was 23 ^{0}C and relative humidity in room was 39 % RH. Syloid was used as the desiccant dust treatment at an application rate of 0.3 g/m^2. Dry ice was used as CO_2 source to stimulate movement in the bed bugs. The average evaporation speed of the dry ice (±SE) for this experiment was 0.34±0.02g/min (Range: 0.28-0.41 g/min).

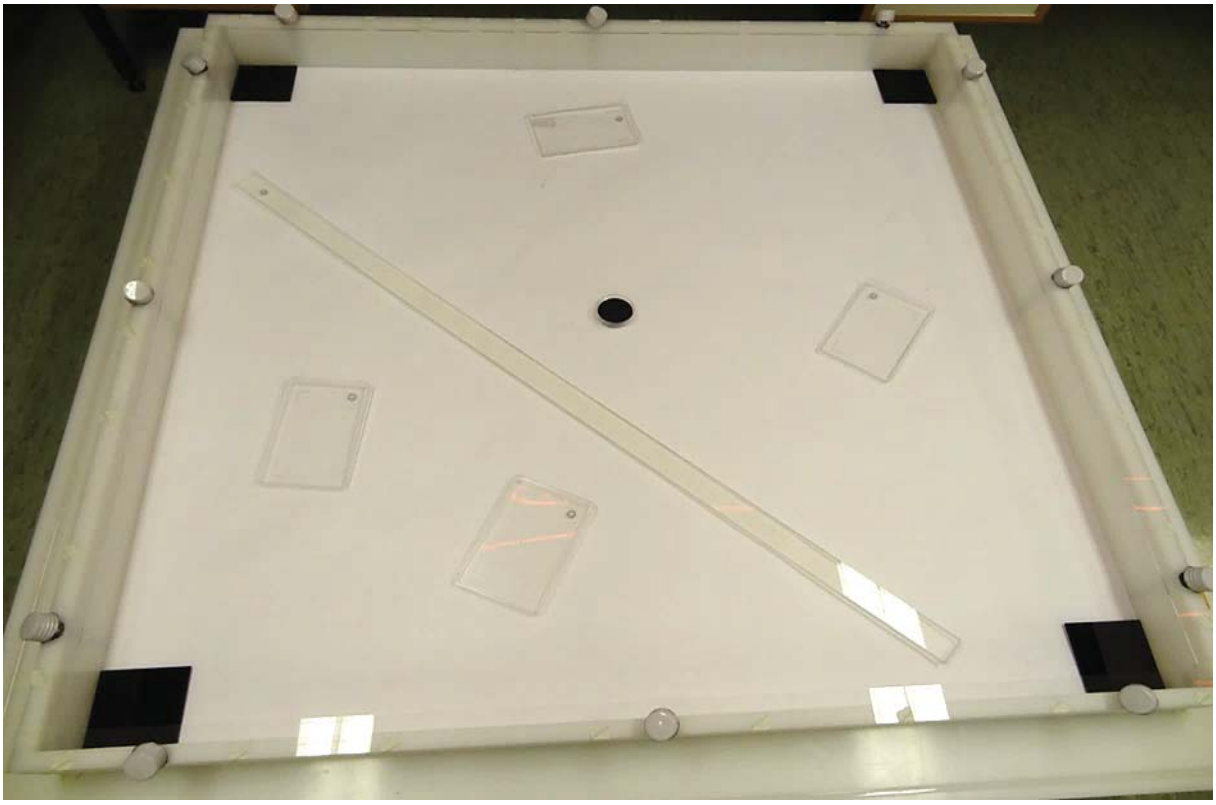


Figure 5. Arena field simulation setup with added obstacles and hiding places.

Protocol: The general protocol in this experiment was the same as in the arena studies with the following exceptions. To allow a longer and possibly more complete acclimation to the arena, bed bugs were blood fed and placed into the arena immediately after feeding. These bugs were given 6 days to acclimate and establish themselves in the harbourages. The addition of nymphs was to simulate a more natural population in the arena. The arenas were treated six days after feeding, however the bed bugs were collected from the arenas 3 days later (Day 8 after feeding) because 3 days is a realistic close down time for a hotel room or the time to leave bedrooms vacant between treatments. Surviving bed bugs were placed in the climate chamber and

mortality was recorded every day up to 20 days post feeding. In this experiment, the Syloid dust was only distributed into the centre 0.25 m^2 of the arena to simulate a partial treatment, which is expected to occur under field conditions as it is not possible to treat every location bed bugs may hide. This provided the bed bugs with a 38 cm wide corridor of untreated space along the walls. To look at the effect of CO_2 this protocol was followed from Day 6 to Day 8 after feeding. For experiments without CO_2 an empty Petri dish was placed into the arena 60 minutes after videoing was started and the Petri dish left for 30 minutes. For the experiments with CO_2, a Petri dish with dry ice was placed into the arena 60 minutes after videoing started and then left for 30 minutes. Videoing lasted for 2 hours from 11:30 to 13:30 during day time and from 19:00 to 21:00 for the night time, from Day 6 to Day 8 after feeding. This procedure provided three daytime videos and two night time videos per arena. The addition of night time videoing was to examine if CO_2 would stimulate more activity at night time compared to day time, and to measure the spontaneous night time activity. When the videos were examined, nymphs were excluded. The arenas were cleaned as above at the completion of the experiments.

Treatment: To measure the effect of CO_2, one arena field simulation with CO_2 and one without CO_2 was undertaken. The dust treatments were either combined with an empty Petri dish or a CO_2 activation source, both for day and night time videoing from day 6 to day 8 after feeding. Every arena had five males and five females, ten 3^{rd}-5^{th} instar nymphs (later instar nymphs) and ten 1^{st}- 2^{nd} instar nymph (early instar nymphs). The arenas were treated with Syloid dust from Day 6 to Day 8 after feeding to examine how a shorter treatment would influence the results. This gave a total of 12 replicates equally divided between the two different control approaches. Bed bugs were gently prodded for a response before videoing to determine if they were dead.

Experiment 4 - Field test

The aim this experiment was to test whether Syloid dust can be used to eradicate bed bugs and if combined with CO_2 would increase bed bug eradication success in an authentic field situation.

Two student accommodations facilities were used for field experiments, which typically represents natural bed bug environments.

When bed bugs were reported in a room, it was inspected along with neighbouring rooms to confirm the infestation. Nine rooms were single bedrooms in a hallway with a shared kitchen and bathroom. Two rooms were apartments consisting of a bedroom, living room/kitchen and a bathroom. In the treatment where CO_2 was used, there were four bedrooms of 12.0 m^2 and one bedroom of 11.2 m^2. For the treatment without CO_2 there were three bedrooms of 12.0 m^2, one bedroom of 11.2 m^2 and two bedrooms of 10.9 m^2. Only the bedroom was treated at all sites. To be included in a field test it was necessary that live bed bugs were found and the room had not

received any treatments within the last six months. To assess the bed bug population density, the skirting boards were removed and all observed bed bugs in the room were counted for 5 minutes. The beds were given special attention and bed bugs on and in the beds were counted for 5 minutes and the faecal spotting counted on the bed frame for 1 minute. The bed and room were then vacuumed for 5 minutes each and the contents collected. A 15 L ash cleaner (imported by Clas Ohlson, Norway) was connected to a vacuum (Nilfisk, Nilfisk-Advance AS, Norway) to collect samples from the bed and room. One L plastic containers were used to hold the samples from the vacuuming. The filter of the ash cleaner was cleaned and emptied into the 1 L container. After the sampling was undertaken, furniture such as beds, chairs, tables and curtains were sent to a freezer for cold treatment for seven days or more. Two different freezers were employed, with the following temperatures -22 ^{0}C and -30 ^{0}C. Rooms were treated using 1 g/m^2 Syloid dust which was applied using an Exacticide applicator (Technicide, California) and left for 7 days. The amount of dust used for the treatment was calculated by determine the floor area (in m^2) of the room. Syloid dust treatments focused mainly on locations were there could be bed bugs harbouring such as behind skirtings, along window frames, and locations with bed bug faecal spotting, however the whole room was treated. Four climb-up interceptor traps (ClimUP insect interceptor, Susan McKnight Inc., Memphis) were placed in the four corners of the room to monitor bed bug activity after treatment, and catches where recorded daily for 7 days. After 7 days the Syloid was removed via vacuuming and the contents of the vacuum collected. A digital timer (Assistant, Germany) was used to keep track of the time and a data logger (Tinytag, Precision Technic Nordic, Norway) monitored temperature and humidity in the rooms. The measured temperature during the experiments ranged from 18-23 ^{0}C and relative humidity in rooms ranged from 40-61 % RH. After seven days, the furniture was returned and a Protect-A-Bed (Protect-A-Bed, USA) mattress encasement was installed on the bed to assist detection on the follow up inspection. The students could then move back in. After 10-12 weeks we came back to evaluate the effect of the treatments. Faecal traces were examined for on the mattress encasement to determine if the bed bugs were eliminated. If any spots of faeces were found on the follow up inspection, the treatment was declared a failure, and if no spots of faeces were observed then the treatment was declared a success (Fig. 6).

Figure 6. Sign of bed bugs after 10-12 weeks (failed treatment) to the left and no sign of bed bugs after 10-12 weeks (successful treatment) to the right.

Treatment: All rooms were treated with the same application rate of desiccant dust (1g/m^2), although half of the rooms were provided with CO_2 once per day, while the other half received no activation stimulant (i.e. no CO_2). The CO_2 source was dry ice in blocks of around 600 grams. Between 14:00-21:00, the dry ice was placed on the floor were the bed were situated prior to removal from the room.

Statistical analysis

For aggregation analysis in the dose-response experiment one way ANOVA analyses were employed. To identify which application rates were significantly different a Dunn's test was used. The movement data in the arena studies and the arena field simulation was analysed using paired t-tests. If the normality test or the equal variance test failed, a Mann-Whitney rank sum test was used. A paired t-test was used on the data collected from the field to test for differences between the two groups (CO_2 vs no CO_2). If the normality test or the equal variance test failed, a Mann-Whitney rank sum test was used. For mortality tests, the Kaplan–Meier product-limit method was employed. The program JMP – statistical discovery, version 8.0.1 (SAS institute, Cary, NC, USA) was used for mortality tests and Sigma Plot 12.3 (Systat Software. Inc.) was used for movement tests.

In the dose-response experiment, the 3 g/m^2 application rate of Syloid, one Petri dish with four bed bugs (4 males) was excluded from our aggregation data as one bed bug died after 9 hours.

A significant difference was found in the mortality across sexes in both the dose-response study and in the arena study. For the dose response experiment a significant difference was found in mortality between female, mixed sex adults and male bed bugs both when examining the effect

of Syloid dust and when examining the effect of DED dust (Kaplan-Meier Log-rank tests: Syloid treatment for female vs mixed sex adults vs male, χ^2=8.70, p<0.013; DED treatments for female vs mixed sex adults vs male, χ^2=15.13, p<0.001). In the arena studies treated with Syloid dust a significant difference was detected between the females, mixed sex adults and male bed bugs (Kaplan-Meier Log-rank tests: Syloid treatment for female vs mixed sex adults vs male, χ^2=10.51, p<0.005). It therefore appears to be an interaction between sex, stimulation and dust treatment, but mortality analysis and interpretation of this is believed to be beyond the scope of a master thesis. Based on this, and due to a relatively low number of replicates data was pooled across genders.

In the arena field simulation experiment, only adult bed bugs were used for movement data as the nymphs were too difficult to observe on the videos. Also only limited analysis on the mortality on the nymphs was undertaken in this experiment. Strangely 3rd-5th instar nymphs had a mortality of 31.7% (19/60) in arena field simulation without CO_2 and 21.7% (13/60) in arena field simulation with CO_2 before the arena was treated with Syloid. All of these nymphs had a hole on the abdomen (Fig. 7). The mortality observed with the nymphs was probably due to problems associated with handling and traumatic insemination. In hindsight, the nymphs were also probably in the process of moulting after being fed 6 days before treatment and thus remained inactive.

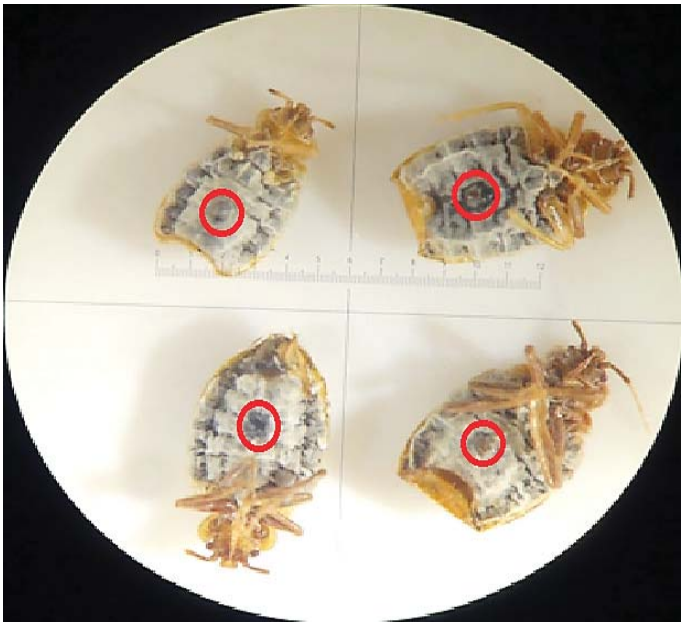


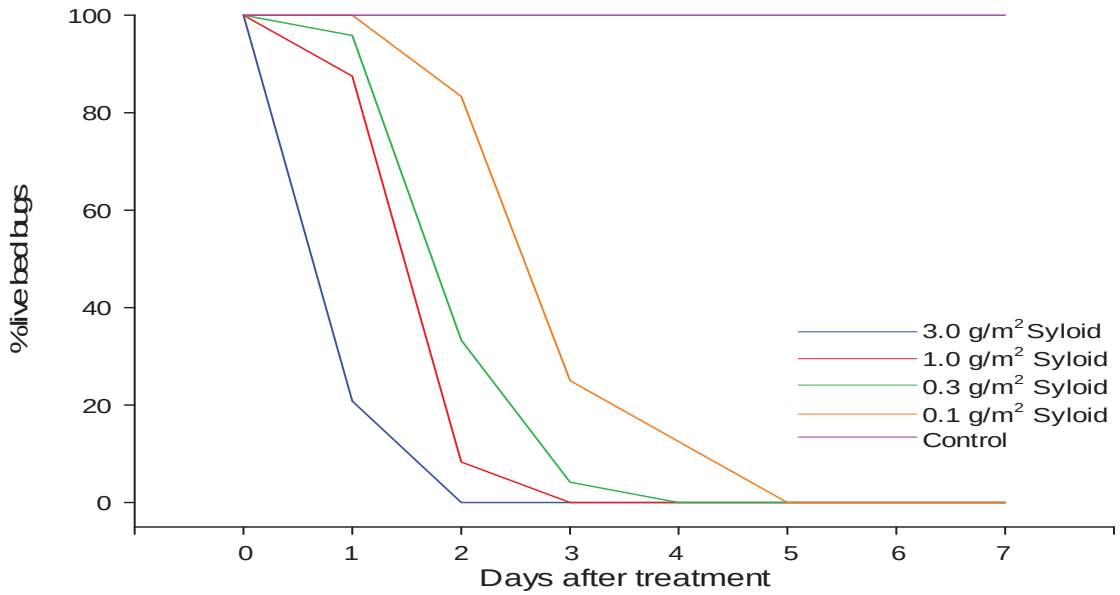
Figure 7. 3rd – 5th instar nymph with hole in abdomen.

Results

Experiment 1 – Dose-response

Mortality

Syloid dust killed all the bed bugs in five days or less, and the time to achieve 100% mortality decreased with increasing application rates (Fig. 8 upper graph). A threefold increase in application rate reduced the time it took to kill all bed bugs with approximately one day. All application rates of Syloid dust were significantly different to each other in terms of mortality (Kaplan-Meier Log-rank tests: 3.0 vs 1.0 g/m^2, χ^2=21.04, p<0.001; 1.0 vs 0.3 g/m^2, χ^2=5.14, p=0.023; 0.3 vs 0.1 g/m^2, χ^2=12.20, p<0.001). DED dust only killed all bed bugs at 3.0 and 1.0 g/m^2 application rates (Fig. 8 lower graph) and there were no significant differences in efficiency between these two highest application rates (Kaplan-Meier Log-rank test: 3.0 vs 1.0 g/m^2, χ^2=1.17, p=0.280). At lower application rates, there was a significant difference between 1.0, 0.3 and 0.1g/m^2 DED dust between mortality (Kaplan-Meier Log-rank test: 1.0 vs 0.3 g/m^2, χ^2=29.30, p<0.001; 0.3 vs 0.1 g/m^2, χ^2=9.20, p<0.002). At 0.3 and 0.1 g/m^2 application rate, DED dust failed to achieve 100% mortality in 7 days. The 0.3 g/m^2 application rate reached 50% mortality in 5 days, while 0.1 g/m^2 application rate never managed to reach 50% mortality in 7 days. No mortality occurred in the control and compared to the control, all desiccant dusts showed a significant mortality effect (Kaplan-Meier Log-rank test: 0.1 g/m^2 Syloid vs control, χ^2=50.20 p<0.001; 0.1 g/m^{2}DED vs control, χ^2=19.82 p<0.001). When the results on mortality between Syloid dust and DED dust are compared for the high application rates of 3.0 and 1.0 g/m^2, there is no significant difference in mortality from start of the experiment too all bed bugs where dead (Kaplan-Meier Log-rank test: 3.0 g/m^{2}syloid vs DED, χ^2=1.80, p=0.179; 1.0 g/m^2 Syloid vs DED, χ^2=1.80, p=0.180). However, at 0.3 and 0.1 g/m^2 application rates of desiccant dusts, Syloid dust killed significantly more bed bugs when compared with DED dust from the start of the experiment too all bed bugs where dead (Kaplan-Meier Log-rank test: 0.3 g/m^{2}syloid vs DED, χ^2=21.92, p<0.001; 0.1 g/m^{2}syloid vs DED, χ^2=45.51, p<0.001). At 0.3 g/m^2 application rate, Syloid dust killed all bed bugs in four days while DED dust only killed 54% in seven days and at 0.1 g/m^2 application rate, Syloid dust killed all bed bugs in five days while DED dust only killed 37% in seven days.



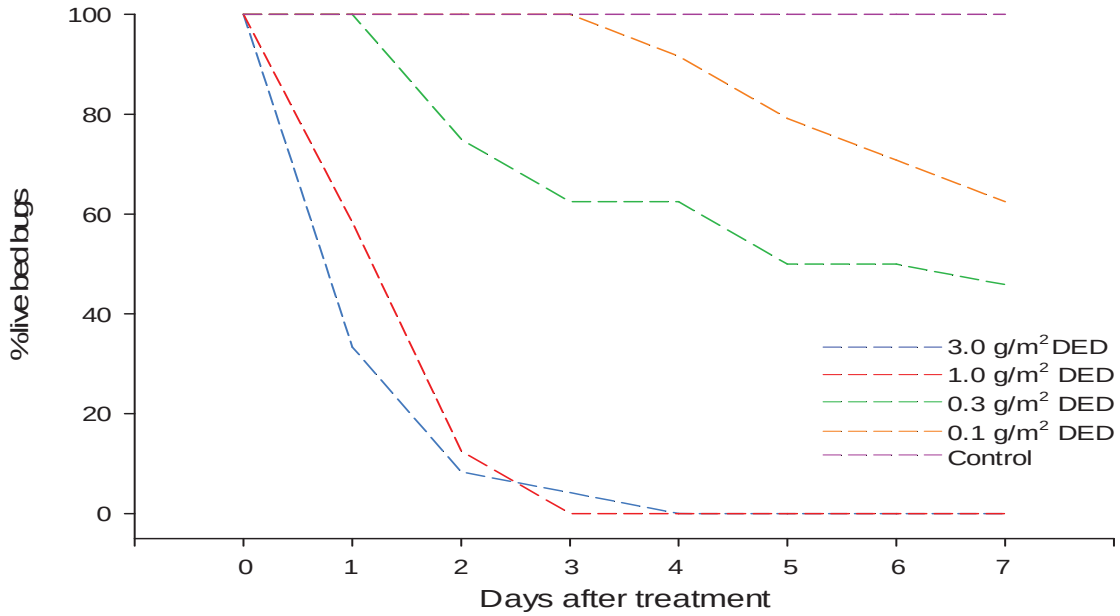


Figure 8. Dose-response with mortality on bed bugs using Syloid (upper graph) and DED (lower graph), n=24.

Aggregation

Different doses of Syloid dust influenced the aggregation behaviour of the bed bugs (ANOVA: H=45.5,Df=4, P≤0.001). Dunn's test showed that the three highest application rates reduced bed bug aggregation compared with the lowest application rate and the control (Fig. 9 left). For DED dust a similar pattern was observed (ANOVA: H=24.3,Df=4, P≤0.001). Dunn's test showed that only in the two highest application rates of DED dust was aggregation reduced compared to the two lowest application rates and the control (Fig. 9 right).

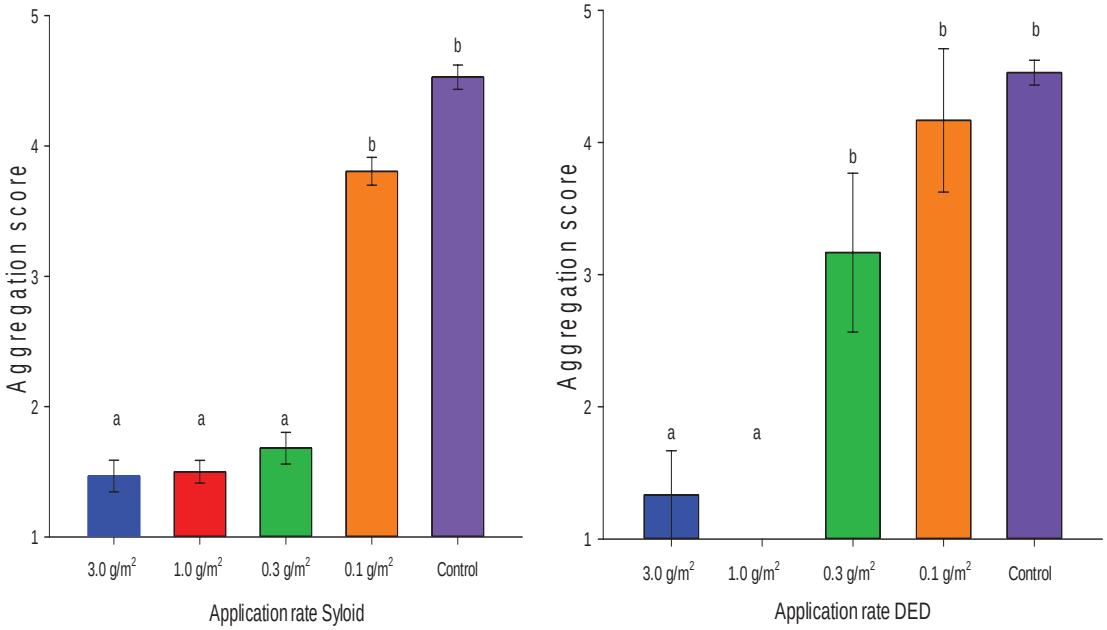


Figure 9. Dose-response with average aggregation for Syloid recorded every hour for 12 hours (left). Dose-response with average aggregation for DED at the 12th hour (right). Mean ±SE; the letter a and b indicates significant difference in aggregation. P<0.05, n=6 for all application rates except 3.0 g/m² Syloid were n=5.

Experiment 2 – Arena studies

Mortality

There was no mortality in the clean arena nor in the clean arena with CO_2, confirming that the untreated arena with or without introduction of CO_2 did not kill bed bugs. There was no significant difference in mortality between Syloid dust treatments with and without CO_2 (Kaplan-Meier Log-rank test: χ^2=1.80 p<0.18). When the clean arena and clean arena with CO_2 was compared against the Syloid dust treatment and Syloid dust treatment with CO_2 there is a significant different in mortality (Kaplan-Meier Log-rank test: clean arena vs Syloid treatment, χ^2=83.98, p<0.0001; clean arena with CO2 vs Syloid treatment with CO_2, χ^2=83.17, p<0.0001). For Syloid dust treatment and Syloid dust treatment with CO_2 all bed bugs died in 5 and 7 days respectively (Fig. 10).

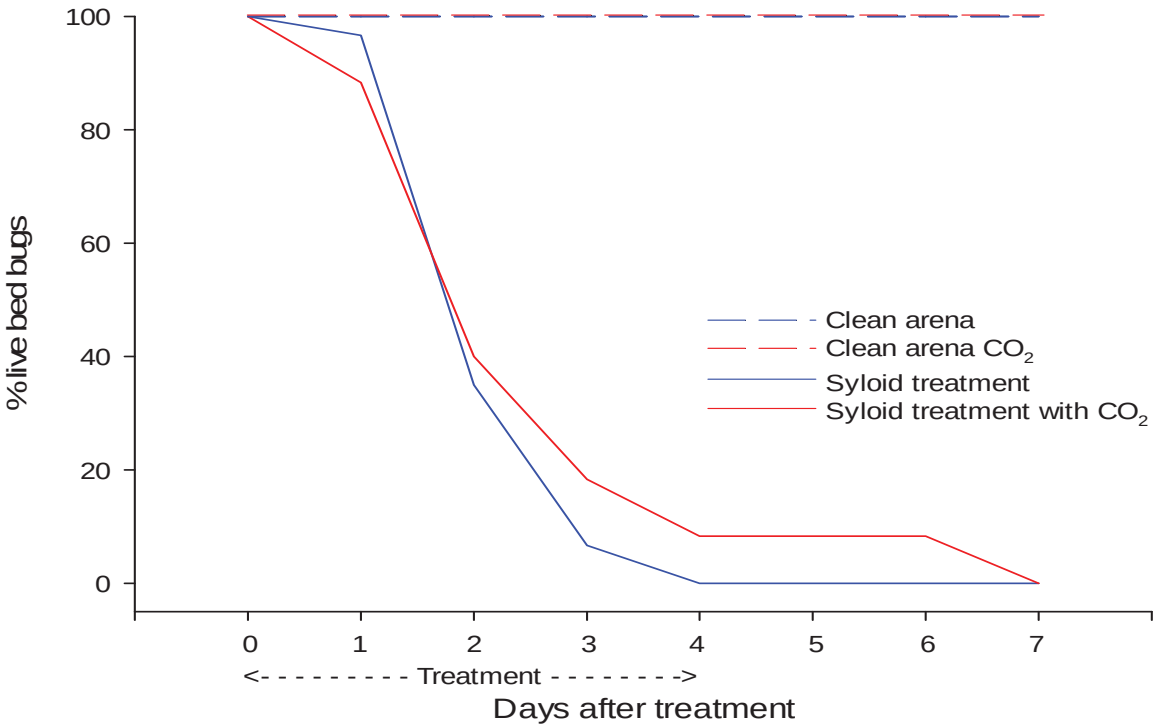


Figure 10. Arena studies with mortality of bed bugs. The Syloid treatment lasted from day 0 to day 4, n=30 for clean arenas both with and without CO_2. N=60 for arenas treated with Syloid dust both with and without CO_2. Mortality was not read at day 5 and 6.

Movement

The introduction of CO_2 increased insect movement in clean arenas. Upon introduction of CO_2, the activity was elevated and became significantly higher when compared to the unstimulated animals after 6-10 minutes (Mann-Whitney rank sum test: Clean arena vs Clean arena with CO_2, T=188.00, n=15, P=0.043, and $p<0.05$ for all subsequent comparisons, (Fig. 11 top graph)). The same pattern was observed in dust treated arenas, but even at the highest level of activation no significant difference was found (Mann-Whitney rank sum test: Syloid treatment vs Syloid treatment with CO_2, T=230.00, n=15, P=0.058, (Fig. 11 bottom graph)). A general increase in movement was also observed when comparing the clean arenas and Syloid dust treated arenas. In Syloid dust treated arenas the overall activity level ranged from approximately 1 to 3, whereas the clean arenas ranged from approximately 0 to 1.2.

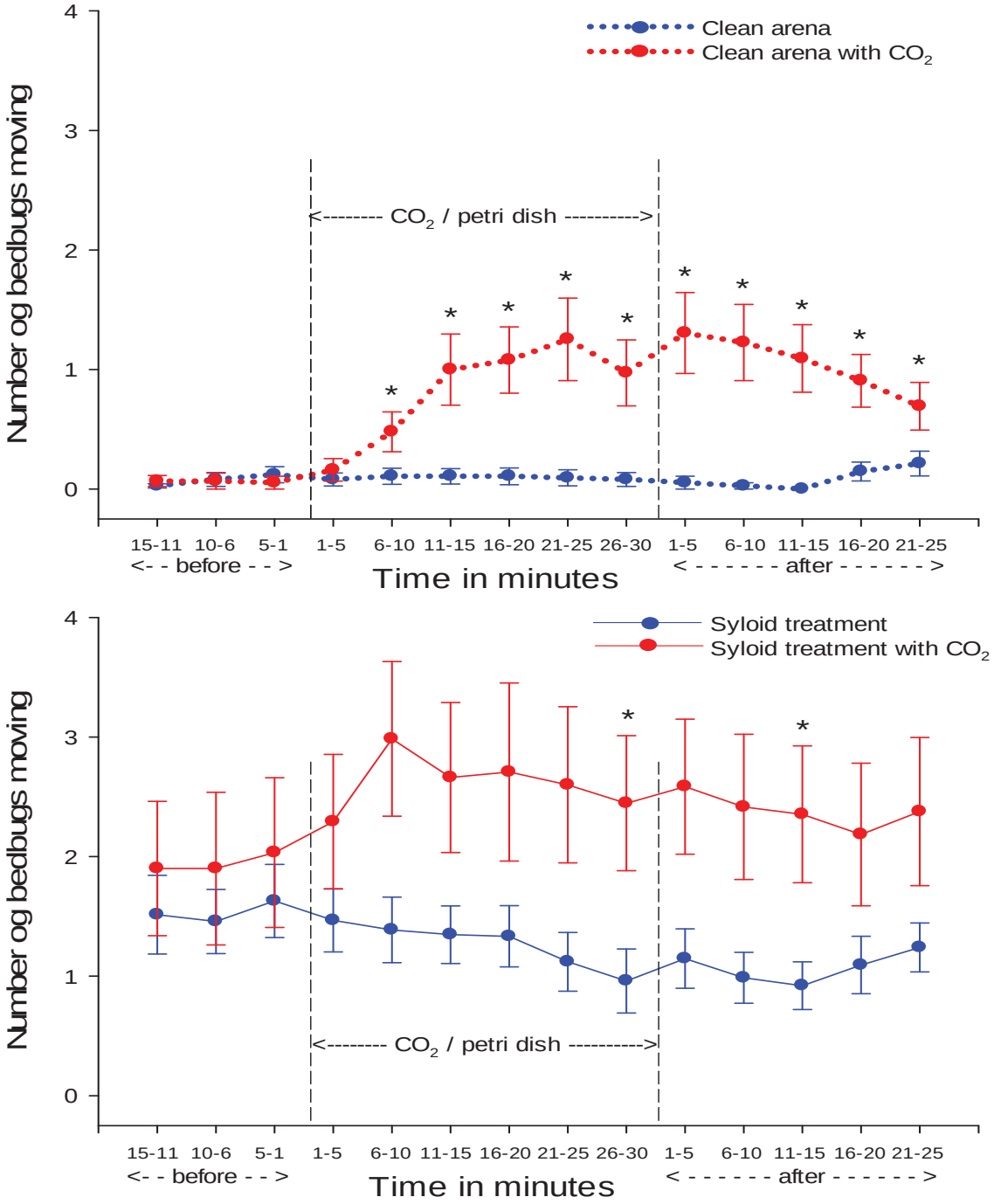


Figure 11. Arena studies with average movement score for bed bugs. Clean arenas top graph and Syloid treated arenas bottom graph. The symbol * indicates significant difference in movement. P<0.05, n=15 for clean with and without CO_2. n=15 for Syloid treatment and n=13 for Syloid treatment with CO_2.

Experiment 3 – Arena Field simulation

Mortality

In the arena field simulation there was a significant lower mortality when compared to the arena study (Kaplan-Meier Log-rank test: arena field simulation without CO_2 vs. arena study no CO_2, χ^2=116.73, p<0.001; arena field simulation with CO_2 vs. arena study with CO_2, χ^2=119.24 p<0.001, (Fig. 10 and 12)). In the arena field simulation partial treatment, adding hiding places and obstacles, and reduced treatment time resulted in only 16.7% mortality for adult bed bugs in 7 days (fig. 12). Using CO_2 in the arena field simulation did not increase the mortality when compared with the arena field simulation without CO_2 (Kaplan-Meier Log-rank test: χ^2=0.11, p<0.74, (Fig. 12)).

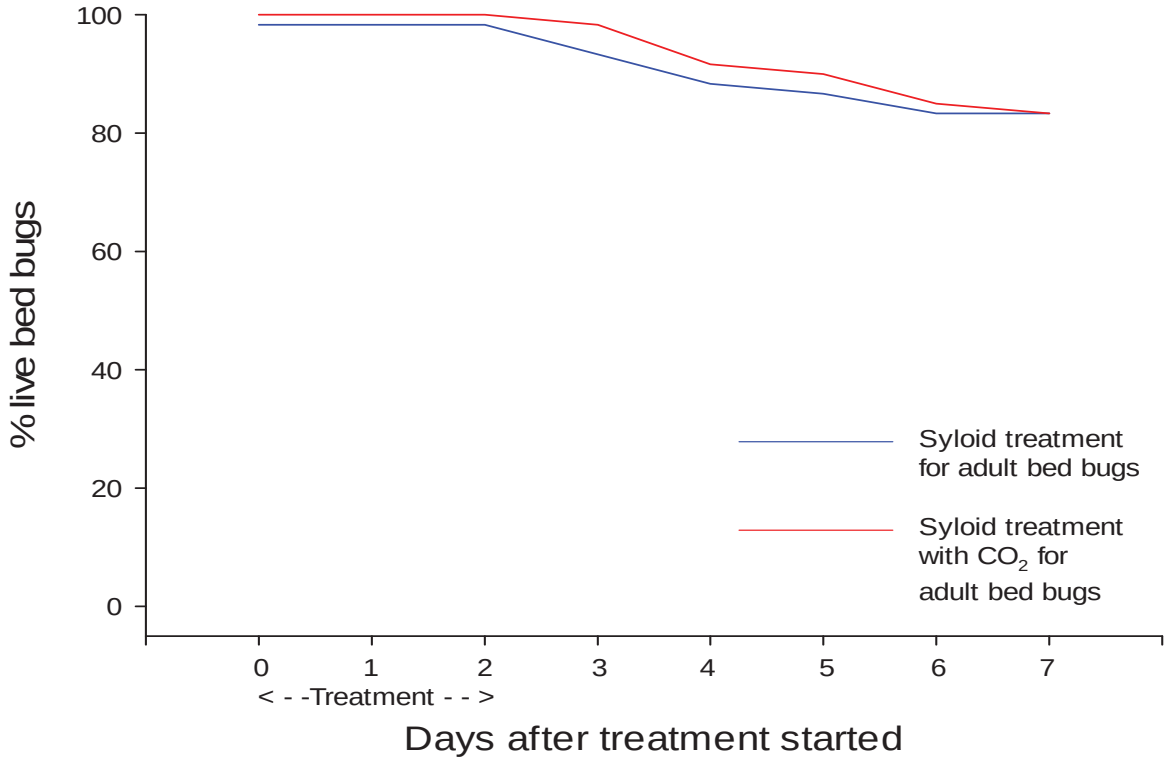


Figure 12. Arena field simulation with mortality of bed bugs when exposed to Syloid. The Syloid treatment lasted from day 0 to day 2, n=60.

Movement

The introduction of CO_2 increased insect movement in the arena field study. Upon introduction of CO_2 at day time, the activity was elevated and became significantly higher when compared to the unstimulated animals after 1-5 minutes after CO_2 was introduced (Mann-Whitney rank sum test: arena field study at day vs with arena field study at day with CO_2, T=240.00, n=18, P<0.001, and p<0.05 for all subsequent comparisons, (Fig 13 upper graph)). The same pattern was seen

upon introduction of CO_2 at night time, the activity was elevated and became significantly higher when compared to the unstimulated animals after 6-10 minutes after CO_2 was introduced (Mann-Whitney rank sum test: arena field study at night vs with arena field study at night with CO_2, T=114.45, n=12, P<0.016, and p<0.05 for all subsequent comparisons, (Fig 13 lower graph)).

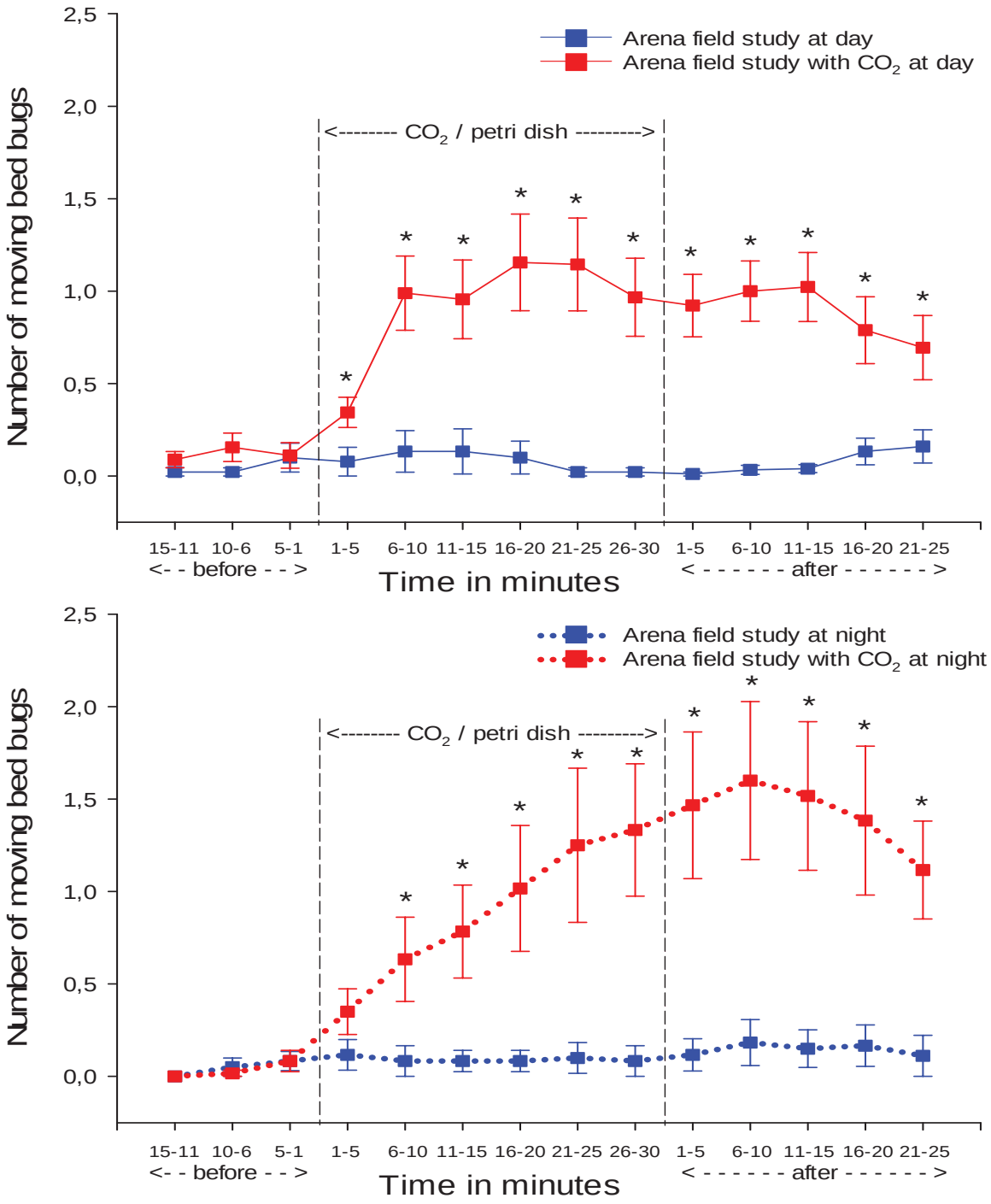


Figure 13. Arena field simulation with average movement score for bed bugs. The symbol * indicates significant difference in movement. P<0.05, n=18 for day (upper graph) and n=12 for night (lower graph) in both tests.

Experiment 4 – Field test

In the field treatments, seven different samplings were undertaken from every room. When the scores from the samplings between the group that received CO_2 and the group that did not receive any CO_2 was compared, there was no significant difference between these groups: Bed bugs found during 5 minute visual inspection of the room (T-test: CO_2 vs no CO_2, t=0.434, n=5, p=0.674), bed bugs found during 5 minute visual inspection of the bed (Mann-Whitney rank sum test: CO_2 vs no CO_2, T=24.50, n=5, P=0.329), bed bugs sampled from the 5 minute vacuum of the room (Mann-Whitney rank sum test: CO_2 vs no CO_2, T=24.00, n=5, P=0.329), bed bugs sampled from the 5 minute vacuum of the bed (Mann-Whitney rank sum test: CO_2 vs no CO_2, T=24.00, n=5, P=0.329), bed bugs caught in Climb-up traps after 7 days (Mann-Whitney rank sum test: CO_2 vs no CO_2, T=24.50, n=5, P=0.329), bed bugs sampled from the room vacuumed after treatment (Mann-Whitney rank sum test: CO_2 vs no CO_2, T=30.00, n=5, P=1.0) and number of faecal spots counted in the bed frame for 1 minute (T-test: CO_2 vs no CO_2, t=-0.203, n=5, p=0.844) (Fig. 14). In all five rooms that received stimulation with CO_2, no faecal spotting was observed on the Protect-a-bed mattress encasements 10-12 weeks after treatment, leading to the conclusion that the treatment was successful. In the six rooms that did not receive CO_2 stimulation, faecal spotting was observed on all the Protect-a-bed mattress encasement 10-12 weeks after treatment, leading to the conclusion of failed treatments.

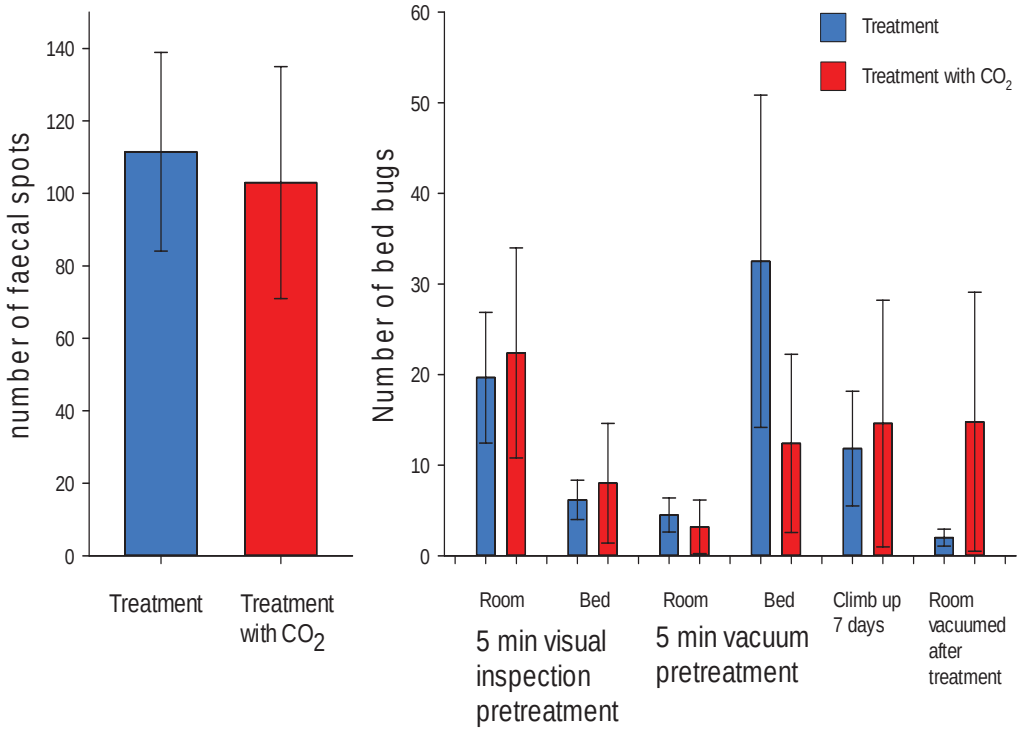


Figure 14. Field test, average collected bed bugs and faecal count. Graph to the right is number of bed bugs counted visually, sampled by vacuuming or via Climb-up traps. Graph to the left is number of faecal spots counted in bed frames in one minute.

Discussion

This study approached bed bug management through a multidiscipline approach encompassing laboratory experiments, arena field simulations, and natural habitat investigations. It was found that Syloid was more efficacious than DED, killing the bed bugs significantly faster and DED failed to achieve 100% mortality at the lower application rates. Both types of dust influenced behaviour. Treating the arena with Syloid dusts resulted in 100% mortality for all bed bugs within seven days. The introduction of CO_2 increased the movement of the insects in the arena, however did not influence mortality. A more natural environment was simulated by introducing nymphs, adding more harbourages, and treating a smaller area of the arena with Syloid dust for a shorter period. This regimen failed as only a minority of the adult bed bugs died. Based on the laboratory trials, it was found necessary to use Syloid at higher application rates, with CO_2 activation and to have the treatment in place in the rooms for seven days in the field trials. This approach seemed to greatly improve bed bug treatment leading to successful bed bug eradication when Syloid dust and CO_2 were combined, and failed treatments when only Syloid dust was used alone.

Utilizing bed bug biology in enhancing control efforts

Bed bugs have evolved to resist dehydration. The thin wax layer of the epicuticule aids in protection against water loss and the insects' aggregating behaviour further helps reduce dehydration (Benoit *et al*. 2007). As they feed approximately once per week and blood is their only source of water, reducing water loss between feeding is a critical evolutionary trait (Usinger 1966). Bed bugs have also evolved to successfully location their host by using heat and kairomones such as CO_2 (Reinhardt & Siva-Jothy 2007). When finding their host, bed bugs have to leave their harbourage location. Using CO_2 to increase catches in traps has been shown to be an effective tool in other investigations (Anderson et al. 2009; Changlu et al. 2009). Other studies have used aggregation pheromones to increase trap catches (Weeks et al. 2011) and alarm pheromones to increase the effect of desiccant dust (Benoit et al. 2009b). In this study we used CO_2 as a kairomon and investigated the effect in combination with desiccant dust treatment. By using desiccant dust to damage bed bugs ability to control water loss (Ebeling 1971) and using CO_2 as a kairomon to draw the bed bugs out from hiding (Reis & Miller 2011), the biology of the bed bug is being used to assist in the eradication of infestations.

Efficacy of desiccant dusts

Laboratory studies have demonstrated that desiccant dust are able to effectively kill bed bugs (Akhtar & Isman 2013; Anderson & Cowles 2012; Benoit *et al*. 2009b; Ebeling 1971; Wang *et al*. 2013) and the investigations herein have shown that Syloid is more efficacious than DED. This

was especially the case for low application rate treatments, where Syloid excelled over DED. It is important that desiccant dust works at low application rates as it is difficult (if not impossible in many situations) to evenly distribute dust treatments in the field to all potential bed bug harbourages (*pers. obs*.). To apply application rates of desiccant dust that effectively kills bed bugs without altering their behaviour or to induce avoidance is important to be successful in eradication attempts. At low application rates with Syloid, bed bug aggregation was observed to be similar to the control, and at low application rates no mortality occurred within the first two days. At high application rates, bed bug behaviour was clearly influenced as they stopped aggregating, although this effect may be a result of stress as bed bugs become close to death. In the arena study we observe an increase of movement in bed bugs before they died. From video analyses it was observed that bed bugs walking in the arenas stopped at the border of the treatment and changed direction away from the treated surface (*pers. obs.*). This increased movement could result in increased risk of spreading the infestation to adjoining rooms. It appears the bed bugs can avoid desiccant dust treated surface and this behaviour may influence the efficacy of desiccant dust. The arena field simulation showed that partial treatment and shorter treatment time greatly reduced the efficacy of the desiccant dust treatment. This shows that it is important to make sure the bed bugs come in contact with the desiccant dust to be successfully in eradication attempts. In Norway, the most commonly used desiccant dust is DED. A product used in the USA that combines a synthetic amorphous silica gel with pyrethroids as a dust formulation is still available for bed bug control even after it is shown that the synthetic amorphous silica gel as the sole active outperforms the combination dust formulation (Anderson & Cowles 2012). Syloid or other synthetic amorphous silica gel should be the preferred option for desiccant dust treatments against bed bugs. The study herein demonstrated that using 1 g/m^2 Syloid for 7 days in combination with CO_2 resulted in a successful treatment against bed bugs.

Stimulation by CO_2

The effect of CO_2 on bed bug movement has also been demonstrated in other studies (Aak *et al*. 2014; Anderson *et al*. 2009; Changlu *et al*. 2009; Reis & Miller 2011; Wang, C. L. *et al*. 2009). However, the combined effect of CO_2 and killing agents had not previously been investigated. In this study it was found that in field experiments, the addition of CO_2 assisted in the eradication of the infestation, while the absence of CO_2 resulted in failed treatments. In the arena studies, no enhanced mortality was observed despite the CO_2 increasing movement of the insects. One of the reasons for this may be that three arenas were employed in the same room and all arenas had their own CO_2 source. This may have made it challenging for the bed bugs to find the direction of the CO_2 source. The limited time CO_2 was present in the arena may not be long enough to achieve

enhanced efficacy of the Syloid dust. The observed night time activity was enough to lead the bed bugs into contact with the Syloid dust when the majority of the arena is treated. This is expected as night time activity last longer than the activity caused by the CO_2 stimuli. As the size of the arenas are small compared to field situations this may limit bed bug behaviour options resulting in movement over the Syloid dust treated surfaces. It would be interesting to investigate the effect of CO_2 if present for an increased time in the arena studies. Under normal field condition it would be expected that CO_2 stimuli from sleeping humans would be present for 7-8 hours or more. The quality of the presented harbourages in the arenas may be sub-optimal, leading to increased movement in search of new harbourages consequently lead to the increased effect of the Syloid dust treatment observed in the arena studies. Compared with the arena studies the field experiment used 20 times the amount of CO_2 per stimulation for 7 days, instead of 3 or 5 days as in the arena studies. By increasing the amount of CO_2, bed bugs were provided with a longer time to react to the stimuli. This was undertaken to compensate for the increased distance to bed bugs harbourages in the field, and the subsequent greater time required for the insect to navigate toward the CO_2 source. By placing the CO_2 source where the bed was originally located may also have influenced the behaviour of the bed bugs in the field, directing them to the location where they previously obtained a blood meal.

Comparing results with other studies

One study done in the field investigated 3 methods (Wang et al. 2013). A dust band treatment consisted of interceptor traps, dust band treatment and an aerosol spray used directly on bed bugs on biweekly inspections. An IPM treatment that was the same as the dust band treatment, except that aerosol spray was exchanged with steam treatment and mattress encasements. The last treatment consisting of only interceptor traps on furniture legs. After 12 weeks only 20% of the apartments in dust band treatments and IPM treatments achieved complete eradication. This study also showed that bed bug numbers increased by 381% 6 weeks after treatments had failed to achieve eradication of the bed bug infestation. All treatment in this study reduced the number of bed bugs in the apartments.

A second study done in the field investigated 3 methods (Wang et al. 2012). A non-chemical method was including installing encasement to mattresses and box spring, applying hot steam and mechanical removal of bed bugs. An insecticides only treatment used 0.075% Temprid SC (imidacloprid and cyfluthrin) and additional treatments of Tempo or Mother Earth D dust. The last treatment was an IPM treatment combining non-chemical and chemical treatments. The apartments was retreated if found necessary during biweekly or monthly inspections. Bed bugs were eradicated from 67% form the non-chemical treatment, 33% from the insecticide only

treatment and 44% from the IPM. In all heavily infested apartments treatment failed, indicating that some bed bugs are more likely to escape treatments in these situations.

A third study done in the field investigated two least toxic integrated pest management programs (Wang, C. et al. 2009). As part of the study all apartments installed encasements on mattresses and box springs and applying hot steam to bed bug infested areas before two IPM methods was evaluated. One group received a diatomaceous earth dust based IPM treatment and bed bug intercepting devices were installed under infested beds and furniture. The other group received a 0.5% chlorfenapyr spray (Phantom) based IPM treatment. All apartments were monitored biweekly and retreated when necessary. After 10 weeks 50% of the apartments in both treatments groups achieved eradication for the bed bug infestation.

A forth study done in the field looked at a reduced-risk insecticide based bed bug management program in low income housing (Singh et al. 2013). This treatment included education, steam, bagging infested linens, placing intercepting devices under furniture legs and corner of rooms, applying Alpine aerosol and Alpine dust (0.25% dinotefuran, 95% diatomaceous earth dust) and regularly scheduled monitoring and re-treatment. With a follow up inspection approximately 6.5 month after the initial treatment 33.33% of the apartment achieved complete eradication of the bed bug infestations. These apartments were considered low infestations at the beginning of the study and heavily infested apartments failed to achieve eradication of the bed bugs.

In all the above studies lack of cooperation from residents and building management staff was pointed out as one of the reasons for treatment failure. Also re-infestation from other untreated apartments was believed to contribute to failed treatments. Leaving a lot of untreated safe harbourages for bed bugs in furniture and residents belongings may have been the contributing factor leading to treatment failure. Letting people stay in the apartment under treatments and thus giving bed bugs the opportunity to feed, may have reduced overall bed bug movements over pesticides also contributing to the reduced effect of the treatments. Newly feed bed bugs show a reduced attraction to CO_2 stimuli for up to 4 days (Aak et al. 2014) which consequently must lead to longer treatment time as bed bugs as passive in their harbourage. Letting residents live in the treated apartments might also have put restrictions on how to apply the insecticide which may have resulted in treatment failure.

In the present study the number of bed bug safe harbourages was reduced to a minimum by treating residents belonging in a freezer. This procedure also left the apartments empty and consequently increasing the area were Syloid dust could be applied. No residents were allowed to use the treated room; eliminating restrictions to application, excluding residence cooperation as a factor and avoiding that the bed bugs could feed. Starved bed bugs are more active when

presented with a host cue (Aak & Rukke 2013), therefor by introducing CO_2 to treatments, multiple activations of the bed bugs should be expected and consequently increase the effect of the desiccant dust treatment. In our study using only Syloid dust lead to 100% failure for eradication attempt, but by adding CO_2 to stimulate bed bug movement 100% eradication of bed bug infestations was achieved. By removing the Syloid dust after treatments this procedure reduces resident exposure to insecticide. The total time spent on site for the field treatments in this study was 2.5 hours per treatment. This includes the treatment, cleaning the apartment after treatment and the follow up visit. Inventory was treated in a freezer on site. The result of the treatment was confirmed by the follow up inspection 10-12 weeks after treatment.

A common protocol for when a bed bug treatment is considered successful should be developed to help clearly determine when a treatment is actually successful or not. This will greatly improve the value of data collected from field studies.

From the laboratory to field conditions; limitations and benefits

In this study four categories of experiments were undertaken. Dose responses in Petri dishes demonstrated the difference between the two desiccant dusts and that desiccant dust influences bed bug behaviour, in particular aggregation. One weakness of this methodology is that bed bugs are unable to avoid the desiccant dust and it limits their natural behaviour by not providing any harbourage. The benefit is that it gives a good measurement on how long bed bugs can live when influenced by different application rates of desiccant dusts.

In the arena studies, bed bugs were presented with harbourages and the option to avoid the Syloid dust. In these studies, mortality was not enhanced by introducing CO_2 to the Syloid dust treatment. The size of the arenas may limit natural bed bug behaviour. The full mortality observed in the arena studies may be a result of this limited space. In the arena field simulation, the larger untreated part of the arena seemed to have provided sufficient untreated areas to ensure that the majority of the bed bugs were not killed, in combination with the shorter treatment time.

Experience from the laboratory studies led to an increase in Syloid dust application rates, amount of CO_2 used, and the time when the rooms were treated in the field experiment. Eradicating bed bugs in a field condition is challenging. One of the problems is that population estimates are hard to do. Also the level of infestations varies greatly between sites and the amount of belongings that needs treatment varies. Another challenge is that after a typical treatment most bed bugs are eradicated and this makes detecting any surviving bed bugs difficult. To overcome these obstacles standardized sampling in the field was applied and re-

inspection 10-12 weeks after treatment to ease the detection of failed treatments. The biggest disadvantage with this treatment is that rooms are required to be vacant for 7 days, and this is not an option in all cases. To keep a room vacant for this time period is the limit for what is practical in a field situation. This is clearly not an option for people that have nowhere else to reside during the treatment process. For the accommodation industry, closing a room for 7 days can be more costly than the treatment. However, placing people at the risk of being bitten when renting rooms, can down grade the reputation of the accommodation provider. This can also increase the risk of spreading bed bugs to new locations and increase the possibility of litigation, if the accommodation provider has knowingly put the customer at risk of being bitten by bed bugs. Reducing the time needed for treatment would greatly benefit the utility of this method. This method has a great potential to be utilized between semesters when student accommodations are empty or for accommodation industry between tenants.

The experiments herein have demonstrated that it is difficult to compare laboratory studies with field investigations. The laboratory is a highly controlled environment and this setting will at best only be a model of the field situation. Laboratory studies are necessary to be able to isolate and understand the specific effect of a treatment excluding other cumulative factors. In field studies, the environment is much less controlled and many other factors may influence the outcome of the treatment, though the method needs to succeed in this environment to be useful.

Conclusions and future directions

Syloid clearly is an effective product for bed bug control that out performed DED in terms of efficacy. CO_2 in combination with Syloid dust led to the eradication of bed bug infestations where Syloid dust treatments without CO_2 failed. This demonstrated that CO_2 enhanced the treatment. Following the protocol of this study, bed bug eradication can be achieved without exposing the residents of the apartment to chemical insecticides as the Syloid dust can be removed after treatment. Therefore, this treatment protocol follows the principles of IPM. A major strength of this study is the demonstrated efficacy of this treatment from laboratory tests to the field trials as requested in the Australian bed bug Code of Practice (Doggett 2013).

The potential of using CO_2 in combination with other treatments should be investigated where it is necessary to lure bed bugs out from harbourages for the treatment to be most effective. Exploiting the effect of CO_2 may reduce the need for further treatments, reduce the time to reach complete eradication and reduce the use of insecticide. Another factor that requires investigating is what happens if bed bugs are able to feed during exposure to desiccant dust. Feeding may reduce the efficacy of desiccant dust treatments and rooms should consequently be kept vacant while treatments are ongoing. Finding out if bed bugs can avoid desiccant dust if

given a choice may help to understand ways to reduce the amount of desiccant dust needed in an application, yet still achieve eradication. Synthetic amorphous silica gels are known to cause irritation to the respiratory tract and the digestive tract of humans. Also the dust may cause irritation to the skin and eyes, so precautions should be taken. Investigations on how to reduce the impact or preferably completely reduce the risk to people living in apartments treated with desiccant dust should be done. Removing the dust completely after treatment, as was done in this study, is one way of achieving this. In the field study, no heavily infested apartments were identified. To test this method in heavily infested apartments would be the ultimate challenge to measure the strength of the methodology.

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