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Tab. 1 An extract from surveys conducted by the Farm Biosecurity Program measuring practice change withi	n
the grains industry at the farm level	

Practice			
	2010	2013	2017
Keep records	59%	82%	89%
Monitor stored products	52%	80%	93%
Clean machinery/equipment coming in property	57%	73%	81%
Control visitor movement on property	34%	47%	54%
Report anything unusual	31%	42%	49%

The latest initiative from the program is a pilot Sentinel Silo project, using both targeted and general surveillance to monitor grain storages, 'ag pantries' and other risk sites and pathways for exotic stored product pests. The surveillance is aimed at strengthening evidence of absence, improving industry participation and knowledge of stored grain pests and surveillance and promoting best management practices.

4. Conclusion

The GFBP is Australia's flagship program for promoting farm biosecurity, with its success encouraging other industries to implement similar extension programs. The focus on biosecurity best practice through industry engagement has seen the GFBP contribute to the safeguarding of grains production and helping to maintain Australia's grain export reputation.

The GFBP is celebrating 10 years of success raising awareness of biosecurity among grain growers and helping the industry respond to serious pest incursions. In March 2018, the program was awarded an Australian Biosecurity Award for its ongoing contribution to Australia's biosecurity integrity.

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A commercial method of controlling bedbugs (*Cimex lectularius*) using CO₂ in dwellings

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Abstract

As a result of withdrawing residual insecticides such as organophosphates and carbamates throughout the world, infestation in *Cimex lectularius* has been dramatically increased in recent years. The ability of this pest to

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starve for three months and its flattened shape of body enables it to hide in tiny holes/cracks or folds, making it difficult to control. Therefore, as a complementary action, fumigating products like textile products and furniture within the dwellings became a practice in Israel. After the conventional treatment of stripping the room, vacuuming, steaming the mattresses and then spraying with a Pyrethroid – a novel method was developed; instead of steaming the mattress and dry cleaning all textile clothes and products, fumigating all movable furniture and textile products with CO₂ inside the dwellings. Based on several scientific papers on the efficacy of CO_2 on the bedbugs, this method has been successfully implemented in Israel. All textile products are inserted into a sealed, low permeability fumigation cube for three days of exposure time at room temperature to reach a calculated concentration of 100% CO₂. Since textile products absorb some of the CO₂, the concentrations quickly drops to about 80%. During the summer of 2017, numerous treatments have been carried out with a 100% success and repeated treatments were not required. This treatment has proved to be a promising method of controlling *C. lectularius* with no need for evacuation of the residents and saving money and efforts in dry cleaning cloths and textile products. It is highly effective against all life stages of the pest.

Key words: Cimex lectularius, CO2 fumigation, bedbug, Modified Atmospheres.

1. Introduction

From ancient history the Greeks and Romans times, to early Jewish and Christian writings bedbugs appear in the literature and folklore of many cultures and countries (Usinger, 1966). Although there are several species of blood-feeding bugs which belong to Hemiptera: Cimicidae which have been persistent pests of humans throughout recorded history, only two species are truly associated with humans. The common bed bug, *Cimex lectularius* L. and the tropical bed bug, *Cimex hemipterus* (Fabricius). Their biology is strikingly similar and most life history traits and behavioral aspects of their biology are overlapping. *C. 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° latitudes (Usinger, 1966).

Bedbugs consume only blood, usually feeding on a mammal (e.g., human, bat) or bird. They need at least one blood meal of adequate volume in each active life stage (instar) to develop to the next stage and to reproduce. There are five nymph stages, and each one may feed multiple times if hosts are readily available. The nymph stage has to feed once to be able to molt into the next stage. The length of the life cycle decreases with temperature up to an optimum of 30°C where it takes 24.2 days to complete (Usinger, 1966). Bedbugs can survive long periods without food and this is also affected by temperature. At 10, 18, 27 and 37°C bedbugs can survive on average for 401.9, 175.6, 43.4 and 17.4 days respectively, if fed once (Usinger, 1966). At low temperature (<10°C) adult bedbugs can survive for a long time without a blood meal. First instars nymphs, when newly hatched, can live up to three months without blood (Usinger, 1966). A fed female lays on average one egg per day (Polanco et al., 2011). It is estimated that bedbugs, at least in theory and under optimal conditions, can lay up to 200 to 500 eggs in a life time (Usinger, 1966).

Adult bedbugs may feed every three to five days throughout their estimated six to 12 month life span. The act of biting a host can cause both physical and psychological discomfort, and can result in local allergic skin reactions to injected salivary proteins (Feingold et al., 1968). Yet, there is no solid evidence bedbugs are disease vectors (Goddard et al., 2009)

When bedbugs have completely engorged, they immediately seek a harborage to hide. They are concealed most of the time; they mate, molt and lay eggs in this cryptic location until they require a blood meal or are disturbed (Usinger, 1966). They prefer harborages 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 behavior protects the insect from detection by their host, increases the chance of mating and helps reduce the loss of water (Benoit et al., 2007). Their favorite sites are wooden frames in box-spring mattresses, behind skirting, wall sockets and cracks in the wall.

The insect prefers to be active at night time, when the host is most likely to be asleep (Romero et al., 2010) and the risk of detection is minimal, but they are also activated by host cues at daytime (Aak et al., 2014). A host seeking bedbug shows positive thermotaxis, the movement towards an up or

down gradient of temperature, but when engorged show negative thermotaxis (Reinhardt and Siva-Jothy, 2007; Reis and Miller, 2011). This results in the bedbugs spending as little time as possible close to their host where the risk of harm and detection is the greatest. Therefore, their biology and cryptic behavior make it difficult to control.

After World War II, widespread use of synthetic insecticides led to sharp declines in bed bug populations in most industrialized countries. By 1997, they were so scarce in the U.S., Canada and Europe that it was difficult to find fresh specimens to use in teaching college entomology classes (Snetsinger, 1997). Many contemporary Pest Management Professionals (PMPs) with years of experience have never seen an active bed bug infestation. During the past 18-20 years, a resurgence of bedbugs has been reported in the U.S., Canada and European countries, Australia and parts of Africa. Infestations have occurred in homes, hotels, hostels, cruise ships, trains, and long-term care facilities (Cooper and Harlan, 2004; Doggett et al., 2004; Hwang et al., 2005; Johnson, 2005).

The increase in trade, the change in pesticides use from residual and more violent once such as organophosphates and organo-clorides, development of resistance to pesticides, the use of second hand furniture and products and the lack of public awareness has led to re-establishment of the pest worldwide, especially in developed countries (Anders et al., 2010; Akhtar and Isman, 2013).

Each country takes its own measures of treating those bedbugs; in Greece, heat treatment for the whole dwelling is applied. Although heat treatment provides no residual effect, there is a potential physical distortion of structures or their contents, as well as flammability risks associated with some kinds of heat sources, may be a concern in particular situations (Usinger, 1966). Moreover, in order to reach the target temperature of more than 47°C, the residents must evacuate their dwellings since the treatment is a 24 h.

In Israel, the conventional treatment consists of several steps; stripping the room, vacuuming, steaming the mattresses and then spraying with a Pyrethroid. After the treatment all cloths, blankets and pillows are either dry cleaned or being washed at a temperature above 60°C. These actions of vacuuming and steaming textile products such as sofas and mattresses requires a lot of working hours which are not always successful. When applying steam treatments, this technique requires practice and care. If the tip is too far away, the steam may not be hot enough to kill all the bedbugs and eggs that it contacts. If the tip is too close, excess moisture may be injected into the treated material, which may lead to other problems (e.g., facilitating dust mite population survival and increase; growth of surface molds). Sometimes the strength of the steam causes the pest to be spread to other hiding places. This time consuming technique of steaming (10-30 seconds) causes mortality of only 84-94% of all stages, if applied properly (Puckett et al., 2013). Also, if the textile cloths are dried in a home dryer, temperatures in the center do not always reach the target temperature of 50°C. Within the army bases, cold treatment is applied. Yet, each of these measures has its own limitations and disadvantages. For example, exposure to low temperatures can kill bedbugs if they are kept cold enough long enough. Bedbugs can tolerate -15°C for short periods and, if acclimated, they can survive at or below 0°C continuously for several days (Usinger, 1966).

Therefore, as a complementary treatment, in order to eradicate the pest from dwellings, a novel method was developed; instead of steaming the mattress and dry cleaning all textile clothes and products, fumigation of all mobile furniture and textile products with CO₂ inside the dwellings is applied. This novel approach is described in this paper.

2. Materials and methods

During the summer of 2017 several commercial treatments were done in several dwellings in Israel.

All textile products were inserted into sealed flexible low permeability fumigation cubes for three days exposure time at room temperature to reach a calculated concentration of 100% CO₂. The surrounding temperatures were 25-30°C. One 13 m³ and three 7.8 m³ flexible fumigation cubes were used to reach, when shrinked, 8, 7.7 and two 5.5 m³ in accordance. The volume of each flexible

fumigation cube was lowered by using a shrink tape around it. After placing the products inside the fumigation cubes a half life-time pressure decay test was applied by negative pressure to ensure gas-tightness of the cubes. Pressure was reduced from 6 mm H₂O to 3 mm H₂O column. Before connecting the pressure hose, an expansion space was left using a perforated plenum. After connecting the pressure hose to the CO₂ cylinder, the cylinder was placed on a scale to measure the amount of the calculated gas at a dosage of 2 kg CO₂/m³. While introducing CO₂, at the top end of the opposite gas introduction side, approximately 30 cm diameter area was left open to enable air to exhaust. Measurements were taken from the top during the introduction of the gas.

3. Results

The results shown in Fig. 1 are measurements of CO₂ taken from the top end of the four flexible fumigation cubes each at a volume of 8, 7.7, 5.5 and 5.5 m³. On average 33 min were needed for the gas purging phase. The amount of CO₂ correlates with its concentration; at the 7.7 m³ fumigation cube 10.3 kg of CO₂ were purged and 85% CO₂ was obtained. The 5.5 m³ fumigation cube which reached 85% CO₂ was flushed with 10.3 kg and the second 5.5 m³ fumigation cube was flushed with 9.5 kg CO₂ to reach 75% CO₂ (Table 1). In the case of the 8 m³ fumigation cube the cylinder was not placed on a scale.

Since the air is being flushed out, measurements were taken from the top end, indicating the weakest point of concentration. Some measurements were taken from the middle (data not shown) indicating an intermediate concentration.

Since all these fumigation cubes were of small volume, at the end of the treatment, after spraying the dwellings, measurements were taken once again, indicating an even distribution of CO₂.

After exposure time of 72 h, measurements were taken again from the top, middle and bottom (only at the 8 m3 cube) of the fumigation cubes (Table 1). Although calculated dosage was of 100% CO₂, it did not reach it due to sorption by the treated textile products.

Table 1: CO_2 concentration (%) in four flexible fumigation cubes at the beginning of exposure time and at the end, after 72 h, the amount of CO_2 (kg) and the time (min) of half life-time pressure decay test.

Volume of the fumigation cube		8 m ³		7.7 m ³		room # 1 5.5 m ³		room # 2 5.5 m ³		
Location of measurment		Bottom	Middle	Тор	Middle	Тор	Middle	Тор	Middle	Тор
[CO2] (%)	T ₀	-	82	86	-	85	72	75		85
	T ₇₂	82.7	73	75.5	84	81.5	80.9	81	81	77
CO2 (kg)					10.3		9.5		10.3	
Time (min) of half time pressure decay test			6		26		24		23	



Figure 1: Increase in CO₂ concentration (%) during purging in four flexible fumigation cubes having the volumes of 8, 7.7, 5.5 and 5.5 m^3 .



Picture 1: *C. lectularius* aggregation in between sofas' folding; adults, nymphs, eggs (left), and a closer view of eggs that hatched (right)-.



Picture 2: Well shrinked fumigation cube (left), and badly placed shrink tape (right).

4. Discussion

Bedbugs (*C. lectularius* L. and *C. hemipterus* F.) are very difficult pests to manage, in part, because of their widespread resistance to insecticides and mostly because of their cryptic behavior (Romero et al., 2007; Zhu et al., 2010). Bedbugs are not limited to sleeping and resting areas such as beds and sofas, instead, literally, anything is susceptible to infestation; from air conditioners outlets, electronics, books, pictures in between sofas (Picture 1) and other household fabrics and equipment. Eliminating bedbugs safely and effectively from these types of items is often more challenging than eliminating bedbugs hiding in cracks and holes in furniture or the structure itself. Therefore either a gaseous treatment is required or physical methods such as heat or cold.

In preliminary laboratory tests by the German Federal Environmental Agency, all life stages of common bedbugs were reportedly killed by constant exposure to very high concentrations of carbon dioxide, at ambient atmospheric pressure, within 24 hours or less (Herrmann et al., 2001). According to Wang et al. (2012), CO₂ fumigation lasting 24-48 h was sufficient to kill all stages of bedbugs at room temperature, depending on the quantity of materials placed in each bag and whether CO₂ was introduced one or two times at the onset.

In these fumigations which were held inside dwellings, in order to ensure both successful treatment and residents' safety, the half life-time pressure decay test is a fundamental step. According to Navarro (1998), in his attempts to correlate gas loss to pressure decay tests, recommended a 5 minutes pressure decay time which were compared with daily CO₂ decay rates of >1% CO₂ daily to obtain successful insect controls (Navarro, 1998). In all of these fumigations, more than 5 minutes were obtained indicating appropriate gastightness for successful treatments (Table 1). Even though pressure tests are not capable of measuring the degree of gas losses through the flexible liner, it still serves as a good indicative measure to predict the degree of gas tightness of the chamber has and whether the fumigation would be successful. It can be understood from Table 1 the significance of this test; there was a decline in gas concentration of 10.5% at the 8 m³ fumigation cube which obtaind only 6 minutes at the half time pressure decay test. Eventhough that, the results show successful treatment with an acceptable decline in gas concentration (Table 1).

The target concentration of 100 % CO_2 was not achieved due to sorption. Also Wang et al., (2012) could not achieve 100% CO_2 when CO_2 was applied from a cylinder and the bags were filled with fabric materials. In previous cases (data not shown) when purging the gas into the flexible fumigation cube, when the pathway was blocked with a textile products such as duvet it absorbed most of the gas and only after its adsorption target concentration was achieved. Therefore, an expansion space must be kept from the purging point of at least 40-50 cm long with a width of 40 cm. Nevertheless, when placing the shrink tape, it is important to wrap the fumigation cube from the very bottom of it to enable air to be flushed out easily (Picture 2). When placing it not from the very bottom, more gas is needed as it was obtained at the 5.5 m³ fumigation cube where 10.3 kg of

 CO_2 were needed to achieve concentration of 77% CO_2 at the end of exposure time instead of 81% CO_2 with only 9.5 kg of CO_2 (Table 1).

Compared with other insects studied, bedbugs are more sensitive to CO₂ fumigation than other urban insects such as oriental cockroach (*Blatta orientalis* L.) (Gannon et al., 2001), and stored product pests (Navarro, 2006). The relatively short exposure time (2 d) makes CO₂ fumigation a promising technique for eliminating bedbugs from infested household items. In commercial use, the recommended exposure time is of 72 h to ensure mortality. After the conventional treatment of stripping the house, vacuuming and spraying with a pyrethroid the residents may return home and stay at home with no risk.

5. Conclusions

CO₂ is an effective alternative of all life stages of bedbugs compared to conventional fumigants for eliminating bedbugs hiding in infested household items such as clothing, shoes, books, electronics, sofas, and other household items. The CO₂ fumigation may be peformed in one of the empty rooms of the house. There is no need to evacuate the residents from neither their dwellings nor the materials to other places in order to fumigate with poisonous fumigation products, to freezing or heating the chambers.

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Mycotoxin prevalence in stored animal feeds and ingredients in Rwanda

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Abstract

Aflatoxins and fumonisins are fungi metabolites produced when climate conditions are favorable. They contaminate feed ingredients when storage conditions are unfavorable. Aflatoxins and fumonisins have a negative impact on animal health and productivity. Humans are indirectly exposed to mycotoxins when they consume contaminated animal source foods from livestock fed contaminated feeds. A total of 3328 feed samples were collected in all 30 district of Rwanda between March and October 2017. Four categories of participants participated in the study (dairy farmers, poultry farmers, feed processors/grain millers, and feed vendors). Feed samples were highly contaminated with aflatoxins but not fumonisins. Average aflatoxin levels were highest in dairy feeds (108.3 μ g/kg) followed by poultry feed (103.81 μ g/kg). Average aflatoxin levels were lowest in samples from feed vendors (88.64 μ g/kg) compared to samples, and recommends year-round surveillance of feed ingredients and mixed feeds for mycotoxin presence. Additionally, more awareness through communication and education needs to be raised among stakeholders in the evolving feed value chain in Rwanda to mitigate the consequences of mycotoxin contamination on public health and animal productivity.

Keywords: aflatoxins, fumonisins, ELISA, value chain

Introduction

Mycotoxins (e.g., aflatoxins and fumonisins) are secondary metabolites produced by fungi. Aflatoxins are produced by *Aspergillus flavus* and *A. parasiticus* while fumonisins are produced by *Fusarium verticillioides* and *F. proliferatum* in favorable conditions. They contaminate crops especially maize, peanuts and cottonseed throughout sub-Saharan Africa (Binder, Tan, Chin, Handl, & Richard, 2007; Perrone & Gallo, 2017; Richard, 2007). Aflatoxins and fumonisins have a negative impact on human and animal health. Human exposure to these mycotoxins is the result of ingestion of contaminated foods (e.g., maize flour, peanut butter), or indirectly from consumption of animal source foods (e.g., dairy products, eggs) derived from animals previously exposed to aflatoxins in feeds. Aflatoxins are classified as carcinogenic substances (IARC, 2002). Fumonisins are associated with neural tube defects, disrupt sphingolipid metabolism and folate transport (Marasas et al., 2004). Fumonisins are also associated with different animal diseases such as Equine Leukoencephalomalacia (ELEM) in horses and Porcine Pulmonary Edema (PPE) in pigs. They are