CAF2020 Paper No. P-9-2-70

Jones K, Morse W, White N, Fields P (2021) Effectiveness of Cryonite system in treatment of stored product insects. Pp. 277-283. In: Jayas DS, Jian F (eds) Proceedings of the 11th International Conference on Controlled Atmosphere and Fumigation in Stored Products (CAF2020), CAF Permanent Committee Secretariat, Winnipeg, Canada.

Effectiveness of Cryonite system in treatment of stored product insects

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Abstract

The Cryonite system turns liquid carbon dioxide to snow at an extremely low temperature (-79°C). The snow is sprayed into cracks and crevices to attempt to lower the temperature of insects below their supercooling point (SCP), the temperature at which they freeze and instantly die. To determine the effectiveness of this alternative treatment in a flour mill setting, Cryonite was applied to cracks in concrete blocks filled with adult insects, *Tribolium castaneum* (Herbst), and flour using two different nozzle types (standard and jet) and at various application durations. Cryonite was used in a grain storage facility to test the efficacy of the system in a real-life scenario. It was found that a 5 s application using the jet nozzle gave 100% mortality within the top 10 cm of the 5 mm wide crack with no flour in crack. The 30, 45, and 60 s applications of the standard nozzle gave mortality rates within the top 10 cm of 79, 97, and 99%, respectively. With cracks deeper than 10 cm, neither the jet nor standard nozzles were effective. Cryonite was ineffective in cracks with flour. For applications in the grain storage facility, the results varied significantly depending on materials and nature of the voids. We recommend that Cryonite not be used as a stand-alone treatment in storage facilities, but rather as a complementary treatment to fumigation or heat treatment.

Keywords: Carbon dioxide, Flour mill, Storage facility, Cryonite, Spray, Freezing

Introduction

After harvest, commodities are stored and processed in a variety of facilities including grain bins, elevators, mills, and warehouses. While food products are being processed for sale, they are susceptible to be attacked by stored-product insects. These insects specialize on feeding on processed plant and animal materials. Historic control of these insects required the use of chemical insecticides, however, through repeated use and misuse of these chemicals, some negative consequences have been discovered (Fields, 1992; Abd El-Aziz, 2011). First, these chemicals are broad spectrum and pose threats to non-target organisms (Fields, 1992). Second, some chemicals, such as methyl bromide, have been proven to be harmful to the environment and are being phased out (Fields and White, 2002; Abd El-Aziz, 2011). Third, insect resistance from over-use or improper use of chemicals has occurred globally (Fields and White, 2002).

Thus, there is interest in finding alternative, non-chemical control methods for stored-product insects. Carbon dioxide can be used to control stored-product insects in two main ways. It can be used as a fumigant either alone or in combination with other fumigants (Boyer et al., 2012). When carbon dioxide gas is pumped into a storage bin or facility, the atmospheric composition changes, reducing the amount of oxygen available, turning the bin into an anoxic environment and inhibiting glycolysis within the insect (Alder, 1994; Boyer et al., 2012; Husain et al., 2017). However, in facilities that are not sealed tightly, it can take a long time to kill pests due to lower concentrations of carbon dioxide. Another way carbon dioxide can be used as a control method is the Cryonite system which produces carbon dioxide snow that freezes insects (CTS Technologies AG, 2008). The system was designed to be used on pests in close contact with humans such as cockroaches, bedbugs, termites, and stored-product insects. The system converts liquid carbon dioxide into a -79°C snow as it is released. This snow is safe to use on a variety of surfaces such as clothing, plastics, wood, metal, and electronics, as it has no residues.

The use of cold temperatures is effective in controlling stored-product insects in a variety of settings (Fields, 1992). The optimal temperature range for most stored-product insects is between 25-33°C (Fields, 1992; Abd El-Aziz, 2011). Temperatures below 20°C cause a halt in development of stored-product insects, and insects eventually die. Lower temperatures cause insects to die faster (Fields, 1992). If temperatures are cold enough, insects will freeze and die instantaneously. This temperature referred to as the supercooling point (SCP), varies from -5 to -30°C depending on species, life stage and acclimation (Fields, 1992). This can be a very effective and an environmentally friendly control method. However, most field studies to date focus on freezing temperature in grain bins over the winter months or in mills that are undergoing freeze-outs. The Cryonite system introduces a new method of using freezing temperatures to control insects in spot/ local treatments within facilities.

For this experiment, we tested the efficacy of the Cryonite system on the red flour beetle, *Tribolium castaneum* (Herbst), using simulated concrete cracks, and in a real grain storage facility. *Tribolium castaneum* has a supercooling point of -12.3°C (Fields, 1992). This is the point at which the body water freezes and becomes lethal (Andreadis and Athanassiou, 2016). Initially, we tested nozzle type, duration of application and width of crack on the temperatures at different depths in the crack. Once this was determined, *T. castaneum* was introduced into the system to determine the effectiveness of the Cryonite system at reducing populations of this stored-product insect. The system was then used in a storage facility to measure temperatures produced by the Cryonite system in voids of varying sizes and composed of various materials.

Materials and methods

Standard nozzle tests without insects

To create a crack that could vary in size, two solid concrete blocks were made, and thermocouples were embedded throughout (Fig. 1). The ten thermocouples could be selectively monitored based on the requirements of the experiment. The temperatures were measured every 1 s. The simulated concrete cracks tests had two main variables: crack size and application duration. The crack widths were of 1, 3, and 5 mm, and the application durations were 30, 45, and 60 s, which resulted in 9 combinations that were tested. The spray handle was held at 50% capacity for each duration.

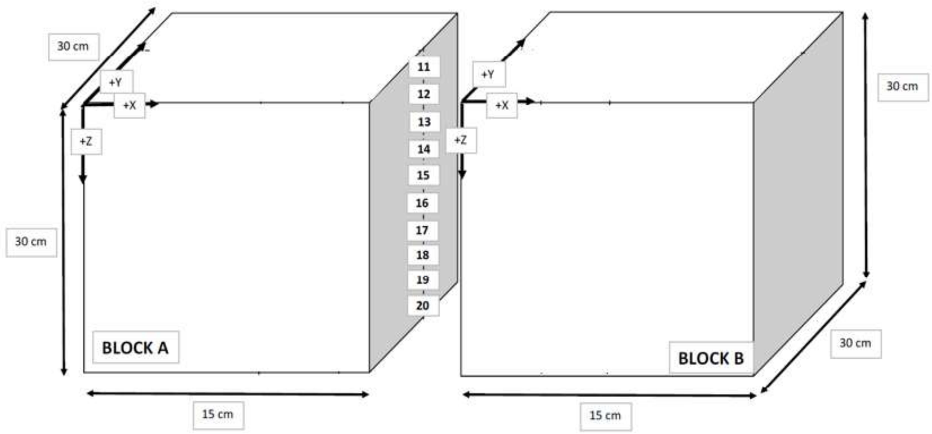

Fig. 1. Locations of thermocouples within the concrete block.

Standard nozzle with insects

This experiment used the same methods as described above with the addition of insects (*T. castaneum*). To keep the *T. castaneum* at a specific location within the crack, 25 adult insects were placed into a small fine mesh bag. Three of these bags were tied to a string at 2, 10 and 20 cm positions. The string was then tied to the top of the block and the insects were placed into the 5 mm crack being held at specific locations along the string. Three replicates were performed for each spray duration. This experiment was completed with insects in the crack and also with insects and flour in the crack.

Jet nozzle with insects

This experiment followed the guidelines recommended in the documentation from CTS Technologies. The jet nozzle was sprayed for a 5 s duration. As in the previous experiment, the crack size was held constant at 5 mm, and the string method with 3 bags of 25 adult *T. castaneum* and no flour.

Application in a storage facility

This experiment followed the same guidelines as the jet nozzle experiment. The Cryonite system, using the jet nozzle was sprayed for a 5 s duration into cracks and crevices. Temperature was monitored in each location. Locations were scouted prior to application so the test would include a variety of crack sizes and materials. Ten locations were selected for Cryonite spot treatments that included bin footings, electrical boxes, wooden beams, vinyl baseboards and other various obstacles that would be hard to access for other control methods. Additionally, five locations in a grain cleaner were selected as this would be a prime location for pests due to the food residues that are often left behind after machine use (Fig. 2). These locations were all sprayed twice to determine minimum temperature reached by the Cryonite jet nozzle.

Results

Standard nozzle without insects

When using the standard nozzle, the lowest temperatures were found in the largest crack sizes. Temperatures increased with depth. In the larger crack sizes, lower temperatures were observed deeper into the concrete block (Table 1). Using the supercooling point for *T. castaneum* of -12.3°C as a point at which the insects would die, the results indicate that the standard nozzle was ineffective for crack sizes of 1 mm. For the 3- and 5-mm cracks, a 45 s application is sufficient to achieve the minimum desired temperature of -12.3°C, and death would be expected under these conditions.

Table 1. Temperatures for locations 11, 12 and 13 (1, 4 and 7 cm depth in crack, respectively) in three crack widths (1, 3 and 5 mm) after three durations of spraying Cryonite (30, 45 and 60 s) with no flour.

Depth in Crack (cm)	Temperature (°C)								
	30 s			45 s			60 s		
	1 mm	3 mm	5 mm	1 mm	3 mm	5 mm	1 mm	3 mm	5 mm
1	-4.8	-33.6*	-31.8*	-13.2*	-23.3*	-37.4*	-9.9	-30.2*	-40.5*
4	13.6	-16.4*	-14.6*	12.5	1.8	-19.8*	11.3	-10.1	-22.3*
7	17.8	-14.2*	-11.6	13.8	3.6	-18.2*	13.9	-9.3	-18.9*

*Temperatures below the supercooling point of *T. castaneum* (-12.3°C), where death should occur (Fields, 1992)

Standard nozzle with insects

When insects were added to the crack, 100% mortality of *T. castaneum* was seen in the top of the crack when Cryonite was sprayed for 45 and 60 s using the standard nozzle. For the 30 s spray duration, mortality was also high at 96% death. Mortality remained high at a depth of 10 cm in the 45 s and 60 s time treatments but mortality was low at depths of 20 cm. Mortality increased with increased spray time ($F=5.52$, $p=0.01$) and decreased within increased depth ($F=20.12$, $p< 0.001$, Table 2).

When flour was added to the crack, no mortality was observed for *T. castaneum* because temperatures below 0°C were not reached at 2 cm below the surface and lower. Mortality was not significantly affected by spray time ($F=1.0$, $p=0.38$) or depth ($F=1.0$, $p=0.38$).

There was a significant discrepancy between the temperature data and the insect mortality. For each replicate, only the thermocouple 2 cm below the surface showed negative minimum temperatures (Table 3), which is inconsistent with the high mortality rates throughout the top 10 cm. This may be due to the method of application, where the snow is getting in contact with the bags of insects, but not the thermocouples directly.

Table 2. Average mortality of *Tribolium castaneum* at three depths (2, 10 and 20 cm deep in crack), three durations of spraying Cryonite (30, 45 and 60 s) into a 5 mm crack with no flour.

Depth in Crack	30 s	45 s	60 s
(cm)	Mortality (%)	Mortality (%)	Mortality (%)
2	96	100	100
10	68	93	97
20	24	53	39

Table 3. Average mortality of *Tribolium castaneum* at three depths (2, 10, and 20 cm) after three spray durations into a 5 mm crack filled with flour.

	30 s		45 s		60 s	
Depth in Crack (cm)	Mortality (%)	Temperature (°C)	Mortality (%)	Temp. (°C)	Mortality (%)	Temperature (°C)
2	0	3.79	1	-7.61	0	-17.45
10	0	22.61	0	20.98	0	20.69
20	0	21.11	0	21.18	0	20.79

Jet nozzle with insects

When using the jet nozzle for only 5 s, 100% mortality of *T. castaneum* was seen up to 10 cm deep in the concrete block. Mortality decreased with increased depth ($F=6.23x10^{30}$, $p< 0.001$, Table 4). Temperatures recorded by the data loggers were, however, inconsistent with temperatures that would cause death for *T. castaneum.* Temperatures at 20 cm deep in the concrete block were too warm to kill *T. castaneum*.

Table 4. Average mortality of *Tribolium castaneum* at three depths (2, 10 , and 20 cm) after a 5 s spray duration into a 5 mm crack.

Depth in Crack (cm)	Mortality after 5 s (%)	Temperature (˚C)
2	100	-21.8
10	100	7.7
20	0	18.9

Applications in a storage facility

For applications in the grain storage facility, the results varied depending on materials, nature of the voids and between applications. Two applications were completed in each location. Temperatures detected varied up to 30°C between the two applications. This suggests that error in aiming of the nozzle likely plays an important role in the efficacy of this system. Interestingly, applications into cracks within wood and within metal provided some of the coldest temperatures and may be more effective in voids of certain materials.

Fig. 2. Locations in Storage Facility with the minimum temperature reached on the two trials: 1. Column with two metal plates bolted together; 2. Inside of drawer on wood cart; 3. Between concrete floor and metal bin footing; 4. Between metal electrical box and drywall; 5. Between concrete floor and metal bin footing; 6. Between a piece of plywood and a wooden block; 7. Behind vinyl baseboard in corner of concrete post and drywall; 8. Inside of plastic clip used to secure small wires; 9. Corner of metal machine where 3 pieces of sheet metal are secured via bolts; and 10. Diamond plate floor access hatch. Locations in Grain Cleaner: 11. Between rubber flap and metal; 12. Between ribbed plastic and metal; 13. Between two pieces of plywood; 14. Along edge of small access hatch to metal chute; and 15. Between layers of sieves, approximately 30 cm away from spray edge.

Discussion

The Cryonite system was able to, under certain conditions, drop temperatures in cracks to below the supercooling point of *T. castaneum* and was able to kill *T. castaneum* adults placed in cracks. The conditions where it worked were in shallow cracks, 10 cm or less, and cracks 5 mm wide or larger. The conditions that it did not work in were cracks of any nature filled with debris or flour, and in thin cracks (less than 5 mm wide) if they were deeper than 4 cm. Therefore, for applications within cracks, the jet nozzle is the preferred method over the standard nozzle. However, this method is only ideal for shallow cracks. While the storage guideline document notes that the jet nozzle should only be used in voids and crack due to the reduced quality of snow produced by this nozzle, the jet nozzle provides the highest mortality rate in the shortest amount of time with the least amount of carbon dioxide.

Stored-product insects all die if frozen, so if SCP temperatures are obtained insects will be controlled. These range from -8 to -20°C (Fields, 1992). Thus, knowing the correct identity of the insect being controlled for is crucial when using this product. The use of ice nucleation bacteria (Fields, 1992) could raise the SCP, making insects more susceptible to this treatment. Additionally, we would recommend that the Cryonite system be used in conjunction with another treatment method, such as a heat or fumigation, where Cryonite be used as an additional treatment in hard-to-reach places. The Cryonite system will not work against insects in cracks filled with flour or dust, so cleaning must take place prior to the use of this control method.

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