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Best Practices for Decontamination of Insects from Container Systems with Decon7

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The global transport and commerce of products present special challenges when infestations of insects and their relatives are detected. Expensive quarantine procedures are costly in time and materials, and currently depend on the greenhouse gas biocidal fumigant, methyl bromide, which is undergoing phase-out from most uses worldwide. In addition to significant environmental concerns, the handling of methyl bromide requires special training, permits and licenses to safeguard human health. Cost-effective and safe alternatives are needed. Decon7 (D7) is a non-corrosive, non-toxic, multicomponent decontaminating product developed to address biological and chemical security threats.

A series of intensive studies were designed to examine the potential biocidal impacts of D7 on multiple orders and families of insects tested in laboratory and on a model macro-invertebrate in a commercial-scale proof-of-concept shipping container system. The goal was to identify best practices for accomplishing complete decontamination of insects from shipping containers.

Small scale bioassay experiments tested both brief exposures (*Exp. 1–2*) of insects to D7, after which they were removed from treated surfaces and placed into recovery chambers, and long-term exposures (*Exp. 3–6*), where insects were left in the sprayed chambers. Food/shelter was provided for the insects, and each test included water-sprayed controls. **Given the broad diversity of species, stages and sizes of insects represented, we conclude that D7 is broadly insecticidal with high likelihood of being used successfully for container cleanup and sanitation with the goal of 100% eradication of invertebrate infestation (Fig. 1; Table 1).**

A commercial-scale proof-of-concept model system was established with ProBox shipping containers (Fig. 2). A series of studies were designed with our model macro-invertebrate species, *Zophobas morio* larvae (Fig. 1), a very large insect with thick integument that has served as an excellent indicator of broad efficacy. ProBoxes (N=12) were infested with 100–200 large larvae and randomly assigned to treatments inclusive of an untreated control. *Exp. 1* tested 24-h exposures to foaming or fogging application methods; *Exp. 2* tested 2–8 h exposures to foaming application (data not shown); and *Exp. 3* tested 2.5–3.5 h exposures to two dilutions/equipment for foaming and full strength fogging. D7 was used at full strength except in *Exp. 1* & *3*, where foam was applied in a 25:1 dilution using an AR Blue Clean Pressure washer; in *Exp. 2* & *3*, foaming was with a commercial unit operating at 65–85 psi.

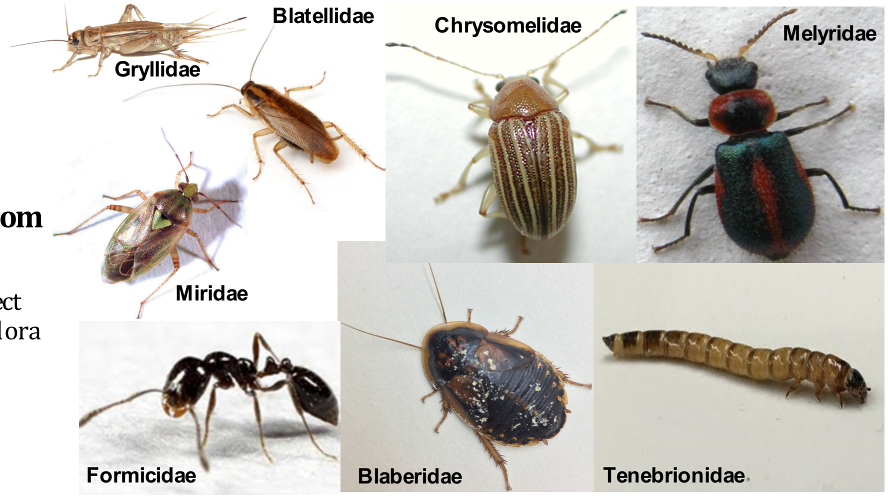

Figure 1. Eight species were successfully killed by Decon7 in small-scale bioassays: Clockwise: house cricket, German cockroach, Colaspis beetle, striped flower beetle, "superworm", Dubia roach, ant, and *Lygus* bug. D7 was capable of killing 100% of test subjects (N=2525) from 8 species, representing 4 insect orders, 7 families, and spanning body sizes from 2–50 mm.

Table 1. Mortality of 8 species of insects when exposed to Decon7 for very brief (Exp. 1–2) or extended intervals (Exp. 3–6). In general, Decon7 killed all individuals of the exposed insects. A total of 4047 insects were assayed.

Exp	Species	Family	Size (mm)	Stage	HAT	M (%)
1	Not determined	Formicidae	S (≤ 2)	Adults	4	80[c1]
1	*Colaspis hesperia* Blake	Chrysomelidae	M (5–8)	Adults	4	100
1	*Collops vittatus* (Say)	Melyridae	M (5–8)	Adults	18	100
1	*Acheta domestica* (L.)	Gryllidae	L (10–20)	Nymphs	1	100
2	Not determined	Formicidae	S (≤ 2)	Adults	1	88[x4]
3	Not determined	Formicidae	S (≤ 2)	Adults	20	72
4	*Blatella germanica* (L.)	Blatellidae	L (13–16)	Mixture	1	100[c3]
4	*Acheta domestica* (L.)	Gryllidae	L (16–20)	Adults	1	100
4	*Lygus hesperus* Knight	Miridae	M (5–8)	Adults	1	100
6	*Zophobas morio* (Fab.)	Tenebrionidae	XL (50–60)	Larvae	1	100
6	*Blaptica dubia* (Serville)	Blaberidae	XL (20–40)	Nymphs	1	100

Exp, *Experiment no.;* ***HAT***, *hours after treatment;* ***M***, *mortality; S, small, M, medium, L, large, XL, very large. The best mortality achieved at the earliest point is shown. Observations were made up to 24 HAT, unless otherwise noted. c1, Control mortality = 13.3%, all ants eventually died in D7; c3, Control mortality 6.6%; for all other species and tests = 0%; x4, Study terminated at this observation period because of excessive control mortality at next interval, 40%. Decon7 killed more than the water controls in each test, T-test; $P < 0.05$*

Figure 2. Commercial storage containers used in these studies, each a 10 ft cube with steel walls & wooden floors.

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Bran meal food was provided in *Exp. 1* & *2* (Fig. 3). In *Exp. 3*, the bran meal food source was raked out to minimize any chance of harborage within the media (Fig. 4). Metal doors were constructed with rubber gasket door sweeps that sealed the doors to the floor, but provided a narrow harborage that was less accessible to directed sprays or fog. At the end of each testing period, all larvae were recovered and classified as dead, "sick", or alive.

These experiments (Table 2) confirmed that high rates of rapid insect mortality is possible with D7 foaming applications (Fig. 5). When conditions are optimized (no pest harborages) and insects are contacted by D7, **100% mortality is possible within 2.5 h of foaming with even diluted application rates**. The lower pressure, more diluted D7 foaming application (25:1) delivered more liquid volume with more opportunity for D7 to reach its intended targets (Fig. 6). All foaming applications were superior to fogging. Fogging is likely more effective against smaller bodied, flying insects, out of reach of other application methods.

Decon7 foam provides users with an excellent, safer and more efficient alternative to more hazardous methyl bromide applications for container arthropod decontamination. The results of these studies underscore the importance of basic sanitation within containers (e.g., removal of soil or other organic debris that could serve as harborages) to achieve complete decontamination with D7 foam, making containers free of all arthropod pests.

Table 2. Commercial-scale shipping containers (N=12) were infested with 100–200 *Zophobas morio* 'superworm' larvae and treated with Decon7 using 3 different application methods: a commercial fogger, a high pressure commercial foamer, and a lower pressure consumer foamer. The foaming treatments were essentially 100% effective when pest harborages were minimized. A total of 5480 insects were assayed over the course of 3 experiments.

Exp	Treatment	HAT	N, Alive	N, Total	Corrected Mortality (%)	
1	Fog, Full Strength	24	354	720	46.5 ±12.5%	A
1	UTC	24	655	706	0 ±0%	B
3	Foam, Full Strength	2.5	1	403	99.8 ±0.2%	A
3	Low Foam, Diluted 25:1	2.5	0	299	100 ±0%	A
3	Fog, Full Strength	3.5	154	192	19.7 ±5.8%	B
3	UTC	6	293	293	0 ±0%	C

***Exp**, Experiment no.; **HAT**, hours after treatment; **N**, total sample size.*
Corrected mortality based on Henderson-Tilton's formula, Henderson & Tilton, 1955
Mean corrected mortalities not sharing a letter within an experiment are significantly different from each other by Tukey's HSD (P < 0.05)

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References

Henderson, C.F. and E. W. Tilton, 1955. Tests with acaricides against the brown wheat mite, J. Econ. Entomol. 48:157-161.

Figure 3. Condition of food at the conclusion of *Exp 1*.

Figure 4. Condition of food medium at the conclusion of *Exp. 3*.

Figure 5. Dead bodies of superworms scattered over the floor at 2.5 h after treatment with diluted foaming of Decon7.

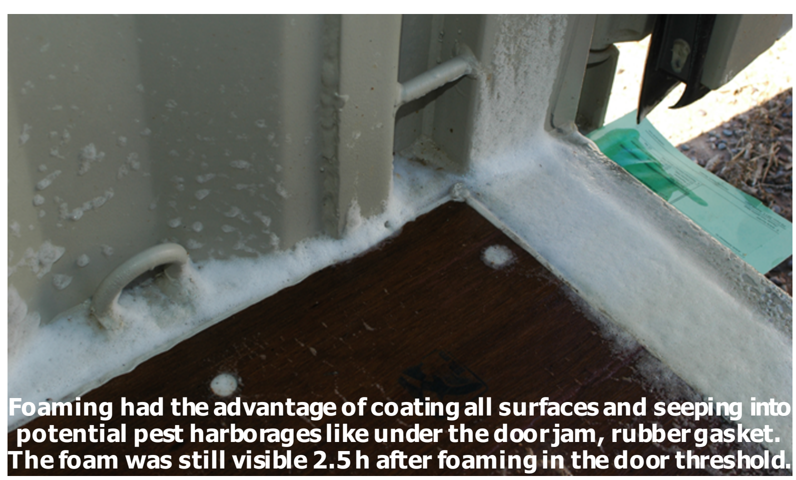

Figure 6. Foaming coats surfaces.