UPDATE ON METABOLIC STRESS DISINFESTATION AND DISINFECTION

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Metabolic stress disinfestation and disinfection (MSDD) is a new process for agricultural products under development at the University of California, Davis[1-3]. MSDD is applied in a closed chamber and consists of a rapid sequence of alternating expansion (low pressure) and compression (high pressure) cycles originating mechanical forces (physical phase) at the end of which a low concentration of a volatile chemical (chemical phase) is applied at reduced pressure. Results have shown that MSDD is effective in controlling microbial and arthropod pests without detrimental effects to the host commodity. In arthropod pests, the magnitude and frequency of the alternating forces helps inducing irreversible damage to vital respiratory structures while eliminating air reserves. An extremely low oxygen environ (anoxia) is achieved rapidly, both externally and internally (cellular level), producing irreversible shifts in cellular chemistry (acidosis). The combined anatomical and physiological stresses is lethal to aerobic microbial and arthropod pests, therefore MSDD is capable of simultaneous disinfestation and disinfection effects. A delay in senescence in fresh fruits has been observed and is being investigated.

An update on the current status of the development and validation of MSDD is given here. The new studies include two insect models, *Drosophila melanogaster* (Meigen) and *Heliothis virescens* (Fabricius) as surrogates for fruit flies and moths, respectively. These surrogate insects were used to determine the extent of MSDD induced anatomical effects leading to mortality. Results of these studies confirmed the potential of MSDD as an alternative quarantine process to chemical fumigation (i.e. methyl bromide) and to irradiation. MSDD processing was also accelerated with a short (~ 2h) chemical phase using removable chemicals (i.e. ethanol vapors).

Disinfestation efficacy using the combined physical and chemical phase (ethanol) of MSDD is given in Table 1, for both *D. melanogaster* and *H. virescens*. Table 2 summarizes the length of time for MSDD processing required for 100% mortality effects for each of the biological stages of *D. melanogaster* and *H. virescens*. According to these results, adult, pupae, and larvae stages of both insect models showed similarly high sensitivity to MSDD, requiring < 0.4 h for complete mortality. As expected, eggs showed a higher resistance requiring an extended (2 to 2.5 h) chemical phase (i.e. with ~ 51 mm of Hg ethanol vapor pressure) to reach 100% control of emergence. Furthermore, eggs of *D. melanogaster* showed a higher resistance than eggs from *H. virescens* (see Table 2).

In preliminary experiments to optimize MSDD prototypes (not shown), the sole application of the physical phase produced nearly 90% control of eggs and 100% control of adult, pupa, and larva stages. However, a longer processing time (12-16 h) was required, a condition considered impractical for commercial uses. Therefore, the addition of the chemical phase, using ethanol in particular, accelerated significantly the lethal

effects with eggs of either insect. With eggs, ethanol induces structural damage of the chorion, softening membranes and facilitating its rupture at reduced pressure, thus shortening the time for effective control of emergence. This is assumed to be due to the partial solubility of eggs lipid membranes in ethanol, which was demonstrated via UV spectrometry, in solutions of 100 eggs of *D. melanogaster* in ethanol and measured over time (up to 12 h)(data not shown). Similar effects were determined for eggs of *H. virescens* suspended in ethanol.

The combined mechanisms of MSDD are affected by the temperature of the pest/host commodity during treatment. This dependence was studied at room (22-23^{o}C) and at refrigerated storage (5^{o}C) temperatures with eggs of *D. melanogaster* and *H. virescens*. At room temperature (23^{o}C), full control of eggs is reached at 2-2.5 h of processing, but it requires ~ 4 h at refrigerated temperature (5^{o}C) (data not shown). Anatomical effects on *D. melanogaster* and *H. virescens* were observed in all biological stages and included body and internal deformations, rupture of tracheal system, expansion of body cavities, loss of internal content (evacuation) and rapid discoloration effects. Examples of these changes in eggs are given in Fig. 1 for *D. melanogaster* and *H. virescens*. Effects on larva stages of *D. melanogaster* are given in Fig. 2. Adults and pupae changes were less evident during experimentation but included external and internal effects. Adult stages showed no obvious anatomical defects except for abdomen deformations, loss of internal content, and the classic fetal position adopted by injured and dead insects (not shown). Changes in pupa stages were less noticeable but included evacuation and discoloration effects (not shown). Except for discoloration effects that were rapidly induced during the chemical phase, all other anatomical changes were originated by the rapid sequence and cycling of expansion (decompression) and compression (pressurization) forces applied during the physical phase (10 cycles).

Finally, design and engineering of pre-commercial systems have began as well as defining process infrastructure for large-scale applications. Results of these studies and the validation of MSDD with other pests will be reported elsewhere.

REFERENCES

1. Lagunas-Solar M.C. (Inventor). “Non-Chemical Disinfestation Method with Induced Metabolic Stress in Modified Environs”. UC Case # 2002-160-1. US Patent Applied.
2. Lagunas-Solar M.C., T.K. Essert, N.X. Zeng, C. Piña U., and T.D. Truong. “Non-Thermal Metabolic Stress Disinfestation and Disinfection Method for Fresh Agricultural Products”. Proc. 2003 Int. Res. Conf. On Methyl Bromide Alternatives and Emissions Reductions, 75-1 to 75-4, November 3-6, 2003, San Diego, CA.
3. Lagunas-Solar M.C., T.K. Essert, C. Piña U., N.X. Zeng, and T.D. Truong. “Metabolic Stress Disinfection and Disinfestation (MSDD): A New, Non-Thermal, Residue-free Process for Fresh Agricultural Products. J. Science Food & Agric. (In Press).

Table 1. Results of MSDD disinfestation effects on various biological stages of *Drosophila melanogaster* (Meigen) and *Heliothis virescens* (Fabricius)*.

Biological Stage	Experimental Replicates	Control Samples Initial	Survival	(%)	MSDD Samples Initial	Survival	(%)
(1) Drosophila melanogaster							
Adults	19	2412	2373	98.4 ± 3.4	2669	0	0
Pupae	18	870	728	83.7 ± 9.8	870	0	0
Larva	19	996	912	91.6 ± 4.8	1046	0	0
Eggs	19	980	746	76.1 ± 24.9	960	3	0.3 ± 0.7
(2) Heliothis virescens							
Larva	3	240	240	100 ± 0	240	0	0
Egg	16	1350	1141	84.5 ± 16.6	1350	0	0

* From: Lagunas-Solar et al., 2005.

Table 2. Summary of MSDD (physical and chemical phases) processing times for 100% mortality of different biological stages of *D. melanogaster* and *H. virescens* *.

	Biological Stages			
	Adult	Pupa	Larva	Egg
Drosophila melanogaster [a]				
Time of Processing (h)	< 0.4	< 0.4	< 0.4	1 -2.5
Heliothis virescens [a, b]				
Time of Processing (h)	< 0.4	< 0.4	< 0.4	1 -2

[a] Results from cumulative experiments at room temperature, with ~ 5,000 each for control and MSDD treated samples for each biological stage. Means between control and treated samples are significantly different statistics ($p < 0.001$) at the 99.9% confidence level.
[b] Experiments with adults and pupae were limited to a few samples (~ 20 each) to verify mortality and were not replicated.
* From: Lagunas-Solar et al., 2005.

Figure 1. Anatomical effects of MSDD on eggs of *D. melanogaster* and *H. virescens*.
Control egg (top left; magnified) shows homogeneous surface. Treated eggs (top right) shows a reticular surface due to dehydration effects. Other effects due to reduced pressure are: loss of content (middle left) and a ruptured chorion (middle right). Discoloration of *H. virescens* eggs (bottom left) as compared with untreated eggs (bottom right). This visual effect shall help regulatory inspections.

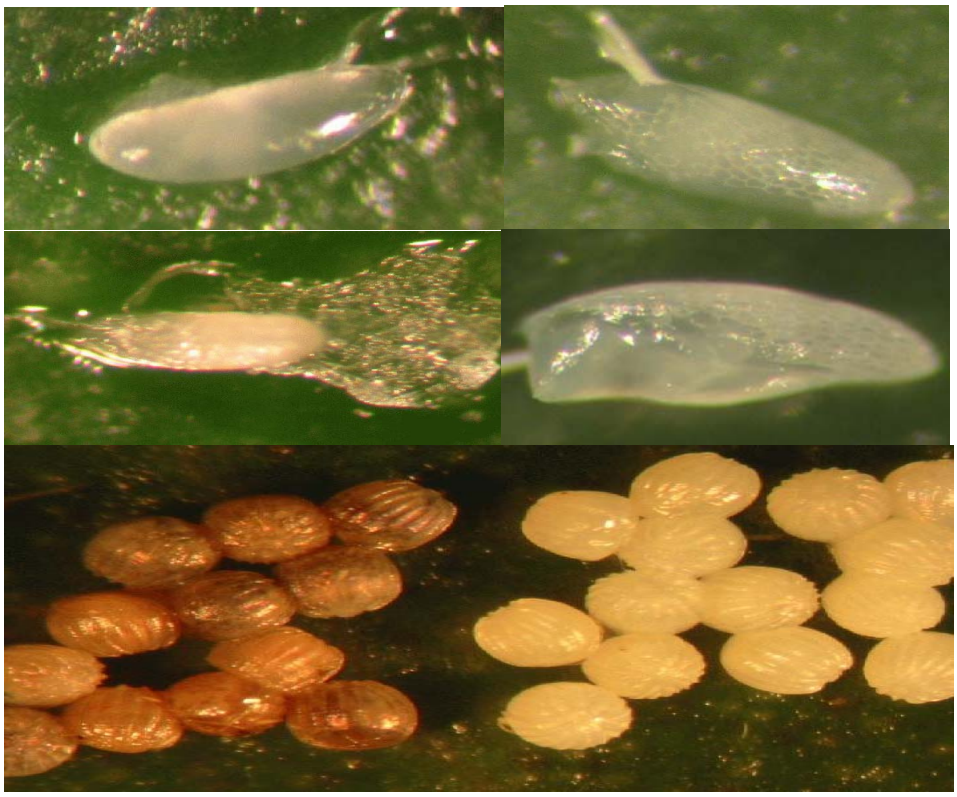

Figure 2. Anatomical effects of MSDD on larva of *Drosophila melanogaster*.
Tracheal system is seen deformed (top left) and deformed and ruptured (top right). Control (not treated) larva is seen on top of contrast media (bottom left) and MSDD treated, dead and discolored larva is observed below (bottom right). Discoloration effects are noticeable immediately after end-of-processing.