NON-THERMAL METABOLIC STRESS DISINFESTATION AND DISINFECTION METHOD FOR FRESH AGRICULTURAL PRODUCTS

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Introduction

A new method for rapid and effective insect & mite control (i.e. disinfestation) that simultaneously induces biocidal and/or biostatic effects, and applicable to a variety of temperature-sensitive fresh agricultural products, is under development at the University of California, Davis[2]. The method is known as "metabolic stress disinfestation & disinfection" (MSDD) and is based on processing fresh commodities for short periods of time (< 24 h; usually 4-12 hours) by creating a modified, inert, gaseous environment (>99.99% modified gas environ with < 0.01% O_2), at above or below barometric pressure, followed by a combination of additional physical and/or chemical stresses acting synergistically. First, MSDD uses a series of rapid, short-timed cyclic pressure differential procedures designed cause mechanical stress in the insect's respiratory system. This procedure effectively uses expansion and compression forces to overcome the ability of insects and mites to establish a reserve of air within collapsible air sacs. Despite the short-time process, the pressure cycles are repeated periodically in order to eliminate (vent) any potentially detrimental gases formed from metabolic processes in fruits and vegetables.

During the disinfestation phase, additional metabolic stress on insects and mites is induced with applications of secondary stress mechanisms including an oscillating or pulsed radiofrequency (RF) field with appropriate electric field intensities. The interaction of RF generated electric fields with insect and mites causes increased oxygen demands as the conductive pests become physically excited trying to react and align themselves to the rapidly changing electric field orientation. This RF effect is induced simultaneously with the establishment of an extremely low-oxygen environment (< 0.01%). Shortly after applying oscillating RF fields (30-60 min), even in the presence of air, RF causes biological injuries and even death (~ 40-60%) in many insects and mites although not at quarantine levels. RF interactions are therefore, an important factor in reaching higher mortality and is an important contributor to the cumulative stress mechanisms used in MSDD.

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[1] Visiting Agronomist (Hortifrut S.A., Santiago, Chile).

[2] Non-Chemical Disinfestation Method with Induced Metabolic Stress in Modified Environs". Manuel C. Lagunas-Solar (Inventor). UC Case # 2002-160-1.

While disinfestation was originally the major objective of this research, effects on microbial growth were also observed in fruit samples, as the presence of spoilage in treated samples was notoriously less than control samples. This was due to the low-oxygen environs as well as the high-concentration of ballast gases (i.e. CO_2) used to reestablish gas pressure equilibrium to prevent or minimize physical or chemical stress on the host commodity. However, under these conditions, the microbial effect was determined to be a biostatic effect as growth resumed once samples were brought into standard air environs.

In order to increase the level of disinfection through biocidal effects, particularly for fruits and vegetables, the modified, reduced pressure oxygen-free environ that initiate the MSDD process, was also used to add volatile chemicals with known disinfection and/or antiseptic properties. The reduced-pressure environs helps to distribute them rapidly and homogeneously within and in between the exposed surfaces of fruits and vegetables. These chemicals were chosen among natural, short lived or easily removed chemicals that would leave no residues. In addition to CO_2 (or N_2 gas) as desirable ballast gases, ozone (O_3) was added from external sources or was generated *in situ* from its atomic (radical) oxygen precursor ($O^{\cdot}$) using RF techniques. In addition, low-level concentrations (< 1 g/L) of H_2O_2 (10 – 30% v/v; m.p. –0.9^{o}C; p_p= 1 mm at 15^{o}C) or ethanol (70-100%; m.p. – 117.3^{o}C; p_p= 40 mm at 19^{o}C) were generated and distributed using their relatively high vapor pressure prompted by the reduced pressure environ. These gases proved to be effective in controlling plant and human pathogens. Therefore, MSDD may also provide opportunities for decreasing or minimizing uses of post-harvest contact pesticides.

The modified gaseous environment aids the method's disinfestation efficiency as well as the gases used also affects respiratory metabolism in insects and mites, reducing its treatment time while simultaneously inducing disinfection effects. Depending on the nature of pest(s) and the properties of the host commodity, MSDD could then be applied solely with combined physical processes or be synergistically combined with one or several natural, transient chemicals leaving no residues. Either way, mortality effects are maximized and accelerated in time allowing the procedure to be completed effectively in less than 24 h (usually 4 to 10 h). However, this time range is highly dependant on the product's tolerance and on the type of infesting pest. Disinfection, on the other hand, can be accomplished in shorter processing times (seconds to minutes).

Results and Discussion

At UC Davis, laboratory-scale testing has demonstrated the effectiveness of MSDD to reach quarantine level disinfestation for many pests including *Frankliniella occidentalis* (Western Flower Thrips, WFT) as tested on iceberg lettuce[3]. A variety of other insects and mites including Fruit Flies (*Drosophila melanogaster*), Ants (*Pogonomyrmex subdentata*), Aphids (*Myzus persicae*),

[3] Infested iceberg lettuce samples were provided by Tanimura & Antle (Data reported in Table 1).

Harlequin Bugs (*Murgantia histrionica)*, and Mites (*Amblyseius cucumeris, Tetranychus urticae*) have also been controlled. A summary of results developed at UC Davis is shown in Table 1 (below).

In all the experiments summarized in Table 1, the remaining insects/mites were severely injured and showed only slight tremors but no or highly impaired physical displacement. It is assumed, although not yet confirmed, that these remaining insects and mites are subjected to irreversible biological injuries with no recovery or yielding biological sterilization effects. It is not known at this time if these injured insects are capable of reproduction. In eggs, eclosion was followed for up to 2 weeks with < 1% estimated emergence.

Disinfection effects have also been demonstrated simultaneously with disinfestation. Under the same conditions for disinfestation, MSDD also controls fungal organisms (i.e. *Botrytis cinerea*, *Penicillium sp*., *Alternaria alternata*, *Rhizopus sp.*,) and bacteria pathogens (*Salmonella sp*., *Escherichia coli* O157:H7, *Staphylococcus aureus*). Reduction effects greater than 5 $\log_{10}$ were measured. Recent disinfection results are summarized in Table 2 (below).

MSDD offers several potential features and advantages:

(1) Time of processing (< 24 h, usually 4-10 h) adequate for most logistical operations in the fresh produce industry.
(2) Disinfestation (longer time) and disinfection (shorter time) effects take place simultaneously. Either effect can be emphasized by proper selection of operating parameters.
(3) Non-thermal process that can be effectively combined with chemical disinfectants or antiseptics of short duration that are easily removed and thus leave no residues.
(4) Potential for sensory and/or physiological changes in the host commodity (i.e. due to anaerobic respiration or fermentation) are largely avoided.
(5) Timing and infrastructure offering practical and economical possibilities for large-scale (pallets) and/or small container operations.
(6) Logistics of MSDD processing would be similar to the 6-8 h methyl bromide fumigation process (including preparation, fumigation, and exhaust cycles). Contrary to methyl bromide fumigation, however, MSDD can be applied at field or at refrigerated temperatures and no post-treatment procedure is required as no obnoxious chemicals are used.

Finally, MSDD prototyping at a laboratory scale has been completed and several research modules able to provide a test bed for each individual stress factors and for any commodity are installed and operational at the Crocker Nuclear Laboratory, University of California, Davis. Future development of this new process is being carried out in collaboration and with the direct participation of the USDA Center for Plant Health Science & Technology and with the involvement of the US and the international private sectors.

Table 1. Summary of UC Davis Disinfestation Results with MSDD (*)			
Insect/Mite	**Life Stage**	**MSDD Time (h)**	**Mortality Effect (%)**
Drosophila melanogaster (Fruit Fly)	Adults Adults Adults	9 10 12	>96% (77/80) >99% (88/89) 100% (55/55)
Frankliniella occidentalis (Thrips)	Adults Pupas Eggs	7 8 8	100% (120/120) >93% (186/200) >93% (156/167)
Myzus persicae (Aphids)	Adults	7	100% (20/20)
Murgantia histrionica (Harlequin Bugs)	Adults	8	100% (30/30)
Pogonomyrmex subdentata (Ants)	Adults	12-14	>98% (43/44)
Tetranychus urticae (Mites)	Adults Juveniles Eggs	6 6 6	>99% (198/200) >90% (205/225) >80% (525/655)
Amblyseius cucumeris (Mites)	Adults	7-10	>98% (294/300)

(*) Some experimental results were combined when process conditions were equivalent. In mortality effect column, percent control is indicated as well as the number of dead species from the initial sample.

Table 2. Summary of UC Davis Surface Disinfection Results with MSDD (*)		
Organisms	**Initial (cfu/mL)**	**Final (cfu/mL) (**)**
Botrytis cinerea	3.0×10^4	No colonies detected
Penicillium sp.	9.0×10^4	No colonies detected
Alternaria alternata	1.2×10^4	No colonies detected
Rhizopus sp.	1.8×10^4	No colonies detected
Salmonella sp.	1.0×10^5	No colonies detected
Escherichia coli O157:H7	1.0×10^5	No colonies detected
Staphylococcus aureus	1.0×10^5	No colonies detected

(*) Using a 6-h long MSD process on surface inoculated Petri dishes and growth media. No sensory effects or chemical changes have been detected for fresh fruits treated with a similar MSD process.

(**) Minimal detection is 400 cfu/mL. Observations made up to 7 days past EOP. All human bacteria pathogen samples were detected at > 10^8 cfu/mL levels during the 7-d incubation and observation period while no colonies were observed in treated samples.