The effect of ultraviolet C radiation on stored-product pests

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Abstract

The potential of using ultraviolet C (UVC) radiation as an alternative treatment and hygiene measure in storage premises was investigated in the laboratory. The effect of UVC on development and progeny production was assessed for pest species of the storage beetles *Oryzaephilus surinamensis* and *Tribolium castaneum*, and the mites, *Acarus siro* and *Tyrophagus putrescentiae*. Photo-reactivation and the effect of indirect exposure were also investigated, as was the effect on spore germination of a mycotoxin-producing fungus *Penicillium verrucosum*. The ED_{95} values for *O. surinamensis*, *T. castaneum*, *T. putrescentiae* and *A. siro* were 96,549, 59,069, 22,014 and 3,802 μJ cm^{-2} respectively, when incubated in lighted conditions. There was an indication of a photo-reactivation effect with *T. putrescentiae*. Limited penetrative ability through substrates was observed at the doses assessed. Complete prevention of spore germination and complete spore destruction of *P. verrucosum* was achieved at 20,000 and 25,000 μJ cm^{-2} respectively. There was no significant difference in the numbers of *O. surinamensis*, *T. castaneum* and *T. putrescentiae* progeny produced by untreated females and females treated with a sub-lethal dose of UVC. However, there was a large degree of variation in the number of progeny produced by individual females. There was a significant reduction in the numbers of *A. siro* progeny produced by UVC treated females compared to untreated females; however, the majority of females died during the incubation period before any eggs had been laid. Practical applications of UVC within a storage environment may lie in the treatment of structural and equipment surfaces, such as conveyor systems. However, cleaning prior to treatment is an important consideration as UVC has limited penetrative ability.

Keywords: Ultraviolet C radiation; Storage beetle, Mite and fungal pests; Structural treatment; Hygiene measure.

1. Introduction

An integrated pest-management strategy is critical for the safe storage of post-harvest commodities. The use of effective hygiene measures and chemical protectants are integral parts of this strategy. However, the number of pesticides currently approved for the protection of stored commodities is very limited. Efficacy may also be affected by the development of pesticide resistant populations. Alternative non-chemical control measures are sought which can be incorporated into this pest management strategy.

In principle, ultraviolet C (UVC) radiation may provide an effective means of combating pest infestations associated with the structure of a building and may serve as a potential new hygiene measure. UVC is short wavelength (100-280 nm) radiation and is primarily used for the disinfection of air, surfaces and liquids from microbial contaminants. The UV destroys the DNA of bacteria and other microbial contaminants, thereby preventing further replication and growth. The use of UVC radiation as a method of pest control has not been extensively investigated due to the perceived risks to human health and the lack of penetration through substrates (Bruce and Lum, 1978). The limited penetration therefore precludes its use as a treatment on bulk commodities. It may, however, offer potential as a surface hygiene treatment in empty stores.

The efficacy of UVC has been previously demonstrated against house dust mites and some stored-product beetle and mite pests (Calderon and Navarro, 1971; Bruce, 1975; Bruce and Lum, 1978; Calderon et al., 1985; Needham et al., 2006; Ghanem and Shamma, 2007; Faruki et al., 2007) with sensitivity varying with species and lifestage (Beard, 1972). It is, however, difficult to make direct comparisons between studies as the level of UV dose achieved is not always stated and UV intensities vary with light sources.

Sensitivity to UVC is determined by the transmittance of surface membranes and the presence of sensitive substrate (Beard, 1972). An increased sensitivity has been demonstrated in the eggs of stored-product moths and beetles (Bruce, 1975; Calderon and Navarro, 1971; Calderon et al., 1985), with moth eggs less sensitive than beetle eggs (Faruki et al., 2007). The effect of UVC on fungal spores is also known to vary among genera, with spores that are thin walled and have a lighter pigmentation being most sensitive (Begum et al., 2009).

Sensitivity has also been found to increase with age, with eggs older than 24 h more sensitive than younger eggs (Bruce, 1975, Calderon and Navarro, 1971; Calderon et al., 1985). Eggs aged between 72 and 96 h undergo a development phase which renders them much more sensitive to UV-induced damage (Calderon et al., 1985), which may reflect changes in the nucleic acid composition or other biochemical and physiological states during embryonic development (Beard, 1972). There has also been a suggestion of a delayed effect of UV radiation on eggs, with mortality increasing when assessed 2 wks post-exposure, compared to when determined by egg hatch (Calderon et al., 1985; Faruki et al., 2007).

Photo-reactivation is an important consideration with UV treatments. Eggs placed in lighted areas after exposure to UVC radiation require a longer exposure period to produce an equivalent lethal effect than those placed in the dark (Bruce and Lum, 1978). Other indirect effects include pheromone degradation (Bruce and Lum, 1978).

The aim of these laboratory experiments was to assess the effect of UVC on the development and progeny production of two species of beetle and mite pests. Photo-reactivation and the effect of substrate were also investigated, as was the effect on spore germination of a mycotoxin-producing fungus.

2. Materials and methods

2.1. Pest species

Laboratory organophosphate (OP) susceptible strains of *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), *Tribolium castaneum* (Herbst) (Coleoptera : Tenebrionidae), *Acarus siro* L. (Astigmata: Acaridae) and *Tyrophagus putrescentiae* (Schrank) (Acaridae) were used. All were reared in the laboratory in constant conditions of 25 ± 2ºC and 70 ± 5% r.h. and 15 ± 2ºC and 90 ± 5% r.h. for the beetle and mites, respectively, without exposure to pesticides. Spores of the storage fungus *Penicillium verrucosum* Dierckx (Trichomaceae) were also used.

2.2. UVC light source

A UVP CX-2000 crosslinker was used to generate the UVC at a wavelength of 254 nm. The crosslinker was calibrated using a UVP radiometer with UVX-25 sensor and found to deliver a light irradiance of approximately 9 mW cm^{-2}. The test samples were placed approximately 9 cm from under the light source.

2.3. Effect of UVC on development

Twenty eggs of each beetle (aged 24 h) and mite (aged 72 h) species were put into separate glass petri dishes (48-mm diameter, 18-mm high) and mite cells (Thind and Muggleton, 1998), respectively. Six replicates were prepared for each UVC dose, including untreated controls. The dishes and cells containing the eggs were placed singly under the light source (without lids) and exposed to five different energy levels (10,000, 20,000, 40,000, 60,000 and 120,000 µJ cm^{-2} for the beetles; 2000, 4000, 8000, 16,000 and 20,000 µJ cm^2 for *T. putrescentiae* and 500, 1000, 1500, 2000 and 4000 µJ cm^2 for *A. siro*). After exposure, the beetle eggs were transferred into glass bioassay jars (120 mL) half-filled with laboratory diet and mite food was added to the mite eggs in the cells. The jars and cells were put into the appropriate controlled conditions and the eggs incubated until adult emergence.

To assess the potential effects of photo-reactivation and substrate, experiments were set up as described above, but the eggs were either incubated in darkness or covered with food (1 g for beetles, 0.03 g for mites) prior to treatment. The effective doses (ED) required to produce 50% and 95% mortality of each pest species were calculated from the dose-mortality data using the probit analysis program PROBIT (version 7). The linear relationship between the logarithm of the dose and the probit of the percentage mortality was estimated using the maximum likelihood method (Finney, 1971).

2.4. Effect of UVC on progeny production

Single virgin pairs of each species were left for fixed periods of time (6 d for beetles, 24 h for mites) to allow mating, but before egg laying commenced. Males and females were then separated and the female insects and mites were put singly into petri dishes and mite cells, respectively. Ten replicates were prepared for each pest species and treatment (including untreated controls). Females were then exposed to UVC for sub-lethal periods of time (2 h for beetles, 12 s for mites). Food was added and test samples were incubated in the appropriate controlled conditions until F_1 development. The numbers of progeny were statistically compared to those produced by untreated females using linear regression with 95% confidence levels.

2.5. Effect of UVC on Penicillium verrucosum

Begum et al. (2009) have found that fungal spores are most susceptible to UVC when spread in a monolayer onto an agar surface. Therefore, agar plates were inoculated with approximately 1,000 spores per plate. The spores were carefully spread around the centre of the plate taking care to avoid the plate edges to ensure that no spores were shielded from the UV treatment. Five replicates were prepared for each treatment including untreated controls. The plates were placed singly and uncovered under the light source and exposed to five different energy levels (5,000, 10,000, 15,000, 20,000 and 25,000 μJ cm^{-2}). The plates were then incubated for 24 h at room temperature (~20°C) and 100 spores per plate were assessed microscopically for germination. A spore was classed as germinated if the germ tube was longer than the length of the spore. Germination counts were repeated after 2 d. The EDs required to reduce spore germination by 50% and 95% were calculated using probit analysis.

3. Results

3.1. Effect of UVC on development

Table 1 shows the ED values required to produce 50% and 95% mortality of each beetle and mite species when exposed to five doses of UVC and incubated in the different conditions. The ED_{95} values varied with species with 96,549, 59,069, 22,014 and 3,802 μJ cm^{-2} calculated for *O. surinamensis*, *T. castaneum*, *T. putrescentiae* and *A. siro*, respectively, when incubated in the light. A significant difference (P<0.05) in mortality of those incubated in light and dark was only observed with *T. putrescentiae*, with ED_{95}s of 22,014 and 14,290 μJ cm^{-2} respectively.

Table 1 UVC doses required to provide 50% and 95% mortality of each pest species.

Species	Incubation periods	ED_{50} (μJ/cm^{-2})	95% fiducial limits for ED_{50}	ED_{95} (μJ/cm^{-2})	95% fiducial limits for ED_{95}	Slope ± S.E.	Chi-square	D.F.	P-value
O. surinamensis	In light	8456	5041, 11671	96549	68750, 167571	1.56 ± 0.22	26.04	23	0.3
	In dark	6726	3668, 9690	91515	64933, 160642	1.45 ± 0.21	24.70	23	0.37
T. castaneum	In light	5678	3032, 8240	59069	44322, 93034	1.62 ± 0.24	28.76	18	0.05
	In dark	4502	2308, 6568	32009	25486, 44986	1.93 ± 0.3	10.72	12	0.55
T. putrescentiae	In light	2358	1747, 2938	22014	16541, 33254	1.7 ± 0.18	18.58	18	0.42
	In dark	669	152, 1299	14290	9444, 32208	1.24 ± 0.26	11.62	18	0.87
	With food	22716	13191, 175380	805255	128460, 1.7×10^{10}	1.06 ± 0.36	55.2	28	0.0016*
A. siro	In light	751	595, 889	3802	2969, 5555	2.34 ± 0.28	18.98	23	0.7
	In dark	760	519, 968	10371	6170, 27538	1.45 ± 0.24	25.94	28	0.58
P. verrucosum	In light	8853	7465, 10149	21393	17833, 28241	4.29 ± 0.53	196.27	15	0.0001*

Limited penetration and effect through food substrates was observed. With *O. surinamensis*, *T. castaneum* and *A. siro*, no probit line could be fitted to the data because the slope was insignificant, indicating no dose response effect. With *T. putrescentiae*, although a line could be fitted through the data, there was a lot of variation between replicates with a significant difference (P<0.05) between the experimental data and the fitted line (Table 1).

3.2. Effect of UVC on progeny production

There was no significant difference in the numbers of progeny from UVC-treated females of *O. surinamensis*, *T. castaneum* and *T. putrescentiae*, compared to those from untreated females (Table 2). There was, however, a lot of variation in the numbers of progeny produced between individual replicates. With *A. siro*, there was a significant (P<0.05) reduction in the numbers of progeny produced by UVC-treated females compared to untreated females (Table 2). However, in the majority of replicates the females had died during the incubation period before any eggs had been laid.

Table 2 Mean numbers of progeny (± S.E.) produced (n=10, *n=9).

Species	Treatment time	Mean numbers of progeny
O. surinamensis	0 (Control)	106.4 ± 21.3
	2 h	83.5 ± 6.4
T. castaneum	0 (Control)	92.8 ± 20.9
	2 h	43.1 ± 22.7
T. putrescentiae	0 (Control) *	20.9 ± 7
	12 s	7.4 ± 2
A. siro	0 (Control) *	21.9 ±6
	12 s *	1.7 ± 1

3.3. Effect of UVC on P. verrucosum

Complete prevention of spore germination and spore destruction was achieved at 20,000 and 25,000 µJ cm^{-2} respectively. An ED_{95} of 21,393 µJ cm^{-2} was calculated (Table 1), however, there was a significant difference (P<0.05) in the goodness of fit between the doses and the fitted line, which may have been due to the limited number of data points, as the top two doses were 100% effective.

4. Discussion

These experiments have demonstrated that UVC is effective at reducing development in a range of storage pests. The ED values varied widely according to species with the mites more sensitive than the beetles. Photo-reactivation was only significantly demonstrated in *T. putrescentiae*. The lack of effect on development when food was present demonstrates the limited penetrative ability of UVC through substrates. In order for treatments to be fully effective, the pests must be in direct contact with the UVC for the required duration or higher doses may be needed. Anything that is likely to shield the pest from exposure, e.g., food particles, dust, debris, cracks and crevices, will affect efficacy.

There was no effect on progeny production with *O. surinamensis*, *T. castaneum* and *T. putrescentiae*. However, there was a large variation in the number of progeny produced by individual females which may have been due to no mating having taken place, variation in egg laying or death of the female during the experiment. The insects also had a tendency to run round the edge of the petri dish, which may have shielded them from the effects of the UVC. It was observed, however, that development was slower in all species following UVC treatment compared to the untreated controls. The significant effect on progeny production in *A. siro* is likely to have been due to the majority of females dying during the incubation period. It is known from the developmental experiments, that *A. siro* is the most sensitive species to UVC and, therefore, a 12-s exposure may have been too long to produce a sub-lethal effect, even though the females were active and moving freely immediately after treatment.

A practical application of UVC to a grain surface has been demonstrated by Hidaka and Kubota (2006). A thin layer of grain, inoculated with micro-organisms, was sterilized as it passed through UVC sources whilst moving along a conveyor system. The time required to obtain a 90% sterilization rate was 6.3 h for bacteria (*Bacillus* and *Pseudomonas* spp.) and 5.6 h for mould (*Aspergillus* and *Penicillium* spp.).

Grain quality was not affected by the UV irradiation. It is, however, difficult to envisage how the entire grain surface would have been treated effectively, as in our preliminary experiments we found that fungal spores appeared shielded from the direct effects of the UVC by the structure of the grain kernel.

Practical applications of UVC within a storage environment may, therefore, lie in the treatment of structural and equipment surfaces, such as conveyor systems. However, cleaning is an important consideration as the presence of food, dust and debris may affect UVC efficacy. The costs and safety implications should also be considered.

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