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IN VITRO RESPONSE OF *PENICILLIUM DIGITATUM* AND *GEOTRICHUM CANDIDUM* TO ULTRAVIOLET (UV-C) EXPOSURE

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Abstract. ***Penicillium digitatum* and *Geotrichum candidum* are among the major organisms causing decay in Florida citrus fruit due to postharvest infection. There exist multiple procedures and many types of fungicides that aid in controlling and eliminating these pathogens. There are existing agrochemicals that successfully aid in controlling these diseases, but there is growing interest in non-chemical methods. Short wave ultraviolet light (UV-C @ 254 nm) has been used in many applications as a method of sterilization. In these trials, culture plates with potato dextrose agar were inoculated with *P. digitatum* and *G. candidum* then exposed to UV-C light for various time intervals. Inhibition of *P. digitatum*. was noted at 40 milliwatt seconds per square centimeter. In the experiment the intensity of the ultraviolet light used at 254 nm was 0.66 milliwatt seconds per square centimeter.**

Fungicides, a major weapon for combating postharvest storage rots, often build resistance and cause concerns about the public's health and the environment (Anon, 1987). As a result of these developments, researchers have sought to find new alternatives to chemical pesticides for controlling postharvest disease of vegetable and fruits (Lu et al., 1987). In this study we inquire ultraviolet light for its effect on post harvest microbes, *Penicillum digitatum* and *Geotrichum candidum*. Their application may be used in future agrochemical techniques. As an important first step, it is necessary to determine the exposure levels needed to control these organisms under conditions optimum for their survival.

P. digitatum (green mold) is a major culprit of much decay in the Florida citrus industry. Spores (conidia) of these fungi are invariably present in the atmosphere of citrus-growing areas, and initial infection of fruit is characteristically via wounds in the peel (Kavanagh and Wood, 1967). Green mold does not spread to the surrounding healthy fruit; there must be some type of abrasion or wound for its entry. These injuries can occur in the groves, during picking, during transporting to the packinghouse, at the packinghouse, or at the consumer's home. *G. candidum* (sour Rot) is also another threat to the fresh citrus industry all around the world. Its method of entry is identical to *P. digitatum*. Pre-harvest infection occurs via injuries caused by insects that may be undetected at picking time (Snowdon, 1990). Post-harvest infection occurs via wounds sustained during harvesting and handling (Casamayor et al., 1983). Spore-laden juices leaking from a rotten fruit can contaminate and infect healthy uninjured fruit (Snowdon, 1990).

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Ultraviolet light is part of the electromagnetic spectrum between visible light and X-rays. This portion of the spectrum is classified into three wavelengths: UV-C (or Short-Wave), from 100 nanometers (nm) to 280 nm, UV-B (Middle-Wave), from 280 nm to 315 nm, UV-A (Long-Wave), from 315 nm to 400 nm. The portion of the UV spectrum between 200-300 nm is considered to be germicidal with a maximum effect at 260 nm. The light intensity is powerful enough to break the outer protective covering of the microorganism and penetrate through the nucleus and deactivate the DNA of the pathogen. To be more specific, the UV-C light forms colvalent bonds between adjacent bases in the DNA. The bonds prevent the DNA from being unzipped for cell replication, therefore, the pathogen is unable to reproduce. Treatment with ultraviolet energy offers several advantages to food processors as it does not leave a residue, does not have legal restrictions and does not require extensive safety equipment to utilize (Yousef and Marth, 1988; Wong et al., 1988).

Long-wave ultraviolet (UV-A), sometimes referred to as 'Blacklight', has long been used in some citrus packinghouses as an aid to grading. Exposure to UV-A has not been determined to be harmful, however, exposure to UV-C can damage skin and eyes. When using UV-C, precautions need to be taken so that workers are not directly exposed to this radiation.

Materials and Method

Cultures of *P. digitatum* and *G. candidum* were obtained from the University of Florida's Citrus Research and Education center at Lake Alfred. Standard petri dishes (3.5 inch diameter) containing potato dextrose agar (PDA) were inoculated with the organism to be tested. Sterile cotton swabs were used to transfer the organism.

For *P. digitatum* the applicating swab was dipped into a suspension containing the organism and then uniformly

Fig. 1. UV-C germicidal light used in the experiments.

Table 1. Response of *Penicillium digitatum* to UVC.

Exposure time	Exposure Mws/sq.cm	Results
10 sec.	6.6	0 - No visible inhibition of sporalation or myclial growth.
40 sec.	26.4	0 - No visible inhibition of sporalation and myclial growth.
60 sec.	39.6	1 - Slight spore and myclial inhibition.
2.5 min.	99.0	3 - Partial inhibition of myclial growth, complete inhinition of sporalation.
5 min.	198.0	4 - Almost complete inhibition, stunted growth of myclium.
10 min.	396.0	5 - Complete inhibition.

streaked in a zigzag motion, until the entire surface was covered with the solution. For *G. candidum,* a single drop of suspension was placed in the center of the plate. The plates were uncovered and exposed immediately thereafter to three 4-watt low pressure UV-C germicidal lights inside a light tight chamber (Fig. 1). The lamps peaked out at 254 nm. With all three UV lamps powered, the output at the surface of the plate was 0.66 milliwatt seconds per square centimeter (mws/cm^2) or 660 micro watt seconds per square centimeter. The intensity of UV-C light was measured with an Extech 40736C Ultraviolet Light Meter. As a point of comparison, during the week of September 8, 2003, noon measurements were taken at Mascotte, Fla, with an average reading on days with clear skies of 22 microwatt seconds per square centimeter.

The UV-C light exposures used in the tests varied from 10 s to 10 min. After the treatments were done the plates were covered and left to incubate for up to 96 h. After enough growth had occurred to distinguish the effects of the various times, mycilial growth, and in the case of *P. digitatum* sporulation, was noted and recorded.

Results and Discussion

After incubation the growth and sporulation was scored on a scale of 1 to 5. 1 representing no effect at all on the growth of the specimen and 5 representing total annihilation of the specimen. Results are recorded in Tables 1 and 2.

Under the conditions of the test, *P. digitatum* was affected by exposure to about 40 mws/cm^2 and was completely killed at 400 mws/cm^2. At 100 mws/cm^2 sporulation was completely inhibited (Table 1) *G. candidum* did not show effect until exposure reached 80 mws/cm^2 but was completely killed at 200 mws/cm^2 (Table 2).

Table 2. Response of *Geotricum candidum* to UVC.

Exposure Time	Exposure Mws/sq.cm	Results
10 sec.	6.6	0 - No inhibition
40 sec.	26.4	0 - No inhibition
2 min.	80.0	1 - Slight inhibition
2.5 min.	99.0	3 - Severe inhibition
5 min.	198.0	5 - Total Kill

The antimicrobial properties that the UV light imposes on these organisms does indeed have some type of a hedonal impact on reducing the reproduction of *P. digitatum* and *G. candidum.* The growth conditions for these two organisms were nearly ideal. A nutrient rich growth medium and ambient temperatures in the mid 70 °F range, provided an excellent environment for growth and reproduction.

One of the methods of preventing postharvest decay is to prevent the decay organism from contacting the fruit. Packinghouse fruit handling surfaces are one of the ways in which organisms may be spread from one infected fruit to others (Brown 1979). The susceptibility of these organisms on various packinghouse environmental surfaces is yet to be determined.

In a packinghouse, with the appropriate safety precautions, this technology may be used as a large scale antimicrobial agent. Use of UV-C as a postharvest treatment for fruits is already practiced in some countries, such as with apples in Chile (Ben-Yehoshua 2003). In a packinghouse, lamps can be suspended over the whole or parts of the processing line to ensure adequate exposure to the UV light. Applying this technology will reduce the total amount of fruit lost to decay.

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