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# PRELIMINARY STUDY OF E-BEAM PROCESSING AS A PHYTOSANITARY TREATMENT AGAINST *Guignardia citricarpa*

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## ABSTRACT

Citrus black spot (CBS) is a fungal disease, caused by *Guignardia citricarpa* and affects practically all citrus species of economic importance, especially sweet oranges. It has become a serious, widespread problem for citrus production in South America. It causes lesions on citrus fruit peel and leaves and can induce fruit drop before maturity. Fruits from citrus areas affected by CBS represent a risk for introduction of this pathogen into new areas. European Community and United States severely limit importation of fresh citrus fruit from those countries were the disease is present. Various treatments have been explored to reduce the risk of introduction of CBS into countries that are currently free of this disease. E-beam processing has been successfully used to inactivate food spoilage microorganisms, including bacteria, yeasts and moulds. Ionizing radiation treatment has been known to extend the postharvest life of many tropical and subtropical fruits. Irradiation is the most recent commercial phytosanitary treatment for fresh commodities. The aim of this study was to assess the effect of electron beam processing on the viability of *G. citricarpa*. Isolated fungi from naturally infected oranges were irradiated with 2.5, 5.0, 7.5, 10.0 and 12.5 kGy using an electron beam irradiator (Radiation Dinamics Co., model JOB-188, New York, USA). Irradiation appeared to be a useful alternative as a phytosanitary treatment to control citrus black spot dissemination.

## 1. INTRODUCTION

Citrus black spot (CBS), caused by *Guignardia citricarpa* Kiely, has become a serious, widespread problem for citrus production in South America [1,2]. The disease is also common in Asia, South Africa, and Australia, but is not known to occur in North America or in Mediterranean citrus-growing areas. The symptoms develop on mature fruit and are characterized as hard spot, false melanose, freckle spot, virulent spot and in Brazil, the recently described cracked spot type [3]. The hard spot symptom is a sunken lesion with black margins and gray centers that often bear pycnidia of the asexual stage of the *G. citricarpa*, Phyllosticta citricarpa (McAlpine) van der Aa. Symptoms on fruit usually develop late in the season, and fruit that are symptomless at harvest may develop CBS during transportation or storage. If symptoms develop before maturity, fruit often drop, resulting in yield loss. Leaf spots may occur as well, especially on those citrus species most susceptible, such as lemon. However, fruit may be severely infected with relatively minor symptoms on foliage. *G. citricarpa* produces ascospores in the decomposing leaf litter on the grove floor [3, 4,5]. Ascospores are produced primarily in the summer and are dispersed by air currents and deposited on leaf and fruit surfaces, where they germinate and form quiescent infections on leaves and fruits [6].

Due to the presence of CBS in many countries in the Southern Hemisphere, the European Community (EC) and the United States (U.S.) severely limit importation of fresh citrus fruit from those countries where the disease is present [7]. Thus, fruit from countries with CBS can no longer be shipped even to EC countries that produce no citrus. Symptomless fruit from regions where CBS is present may develop symptoms during transportation. Exposure of fruit to intense light and high temperatures speeds development of CBS symptoms [8]. In the protocol that allowed shipment of lemons from Argentina to the United States, samples from fruit lots for export were exposed to these conducive conditions prior to shipment to be sure they were free of CBS.

Various treatments have been explored to reduce the risk of introduction of CBS into countries that are currently free of this disease. Several preharvest treatments (such as sprays of copper fungicides) and postharvest treatments (e.g. applications of thiabendazole) did not reduce the development of CBS after harvest. Others were effective in delaying expression of CBS after harvest (sprays of benzimidazoles, applications of thiophanatemethyl, hot water, fruits waxes and refrigeration) but did not prevent lesion development [9].

Ionizing radiation treatment has been known to extend the postharvest life of many tropical and subtropical fruits. Irradiation is the most recent commercial phytosanitary treatment for fresh commodities. Recent developments in the expansion of irradiation facilities in developing countries may prove beneficial to enhance the international trade through overcoming phytosanitary barriers and reducing postharvest losses in fresh fruits [10]. As CBS affects mainly the surface of fruits and leaves, E-beam treatment of fruits could be an effective tool to control and inactivate *G. citricarpa*, considering its high energy and low penetration power.

In this study, E-beam processing was applied to isolated *G. citricarpa* colonies from naturally infected oranges in order to evaluate its application as a useful and effective phytosanitary treatment against this pathogen.

## 2. MATERIAL AND METHODS

### 2.1. Samples

Naturally infected Valencia orange (*Citrus sinensis*) from orchards in Araraquara (São Paulo, Brazil) were courteously supplied by Sucocítrico Cutrale Ltda.

## 2.2. Fungal Isolation and Identification

Lesion fragments of about 5 $mm^2$ were removed from fruits with hard spot symptoms and immersed in 70% ethanol for 1 min, then in a 1:3 v/v mixture of sodium hypochlorite: sterile water for 3 min and rinsed in sterile water for 30s. After drying of the material on sterile filter paper, the fragments were placed aseptically in Petri dishes containing oatmeal (OA) medium and potato dextrose agar (PDA) (Oxoid) with chloranphenicol (0.01% w/v). Plates were incubated for 7 to 10 days at 25ºC. After incubation, typical positive *Guignardia citricarpa* colonies were identified as described by [7,11].

Following to isolation and identification, pieces from isolated colonies of about 25 $mm^2$ were irradiated into sterile Petri dishes with its respective radiation doses. Then, they were placed aseptically into new Petri dishes containing OA and PDA medium and incubated for 7 to 10 days at 25ºC to radiation treatment assessment. Assay was performed three times, each time in triplicate and results were expressed as the frequency of fungal contamination.

## 2.2. Irradiation Treatment

Samples of isolated *G. citricarpa* colonies were irradiated at IPEN-CNEN Electron Accelerator, a Dynamitron Machine (Radiation Dynamics Co. model JOB-188, New York, USA), with 0.965 MeV energy, scan 100 cm and support speed 6.72 m/min. Applied doses were 0 (control), 2.5, 5.0, 7.5, 10.0 and 12.5 kGy; dose rate was 5.58 kGy/s and electrical current 1.09 mA. CTA dosimeters were used for the radiation dose measurement.

# 3. RESULTS AND DISCUSSION

In this study, *G. citricarpa* were successfully isolated from hard spot lesions of naturally infected orange samples. Microbiological analysis carried out both in PDA medium and OA medium, showed that fungi development was faster in the first one compared to the latter. While the use of OA medium allowed an efficient differentiation between pathogenic and non-pathogenic isolates (without a yellow halo around colony), as previously described [7, 11], PDA medium represents an important tool to differentiate between *G. citricarpa* and *G. mangiferae*, an endophytic asymptomatic form.

After E-beam processing, surprisingly, at the lowest radiation dose applied (2.5kGy), fungi viability was totally inhibited. Radiation doses from 5.0kGy to 12.5kGy also inhibit completely fungi development. It suggests that a radiation dose of 2.5kGy would be effective to inactivate *G. citricarpa* in culture.

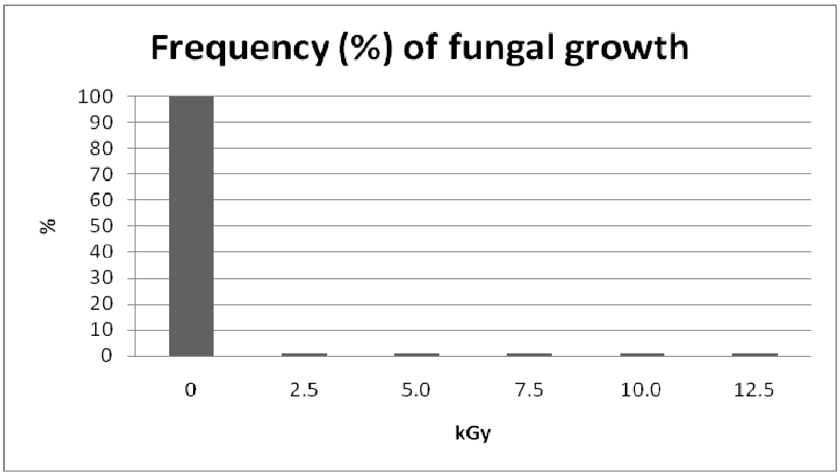


**Figure 1. Frequency of fungal growth of control and irradiated samples of isolated *G. citricarpa* colonies from naturally infected oranges (average of three replicates).**

In our study, *G. citricarpa* was effectively inactivated with a low radiation dose of 2.5kGy. These results probably indicate that genus *Guignardia* would be very susceptible to radiation treatment, at least under the conditions assayed.

At present, the control of this pathogen dissemination is made with the use of fungicides that sometimes does not work properly. Even though irradiation does not return infection itself, it would be a useful tool to prevent fungi dissemination over the world and to enhance Brazilian citriculture exportation to countries free of the disease.

According to [12], a dose of 2 kGy markedly reduces the number of microorganisms present in foods, and higher doses (4–6 kGy) completely inhibit the presence of fungi in foods. These statements are in accordance with our results, which revealed fungi predominantly in those samples treated with a dose lower than 2.5kGy.

As the present work was done during inter-harvest period time, this approach on *G. citricarpa* radioresistance was performed to isolated colonies from naturally infected fruits. It is of great importance to repeat this experiment in naturally infected fruits to determine whether radioresistance of isolated colonies matches to the radioresistance of fungal contamination in fruits and also the irradiation effects on fruit itself. Although it is well known E-beam penetration reaches only some millimeters, this assay will be strongly important to verify sensorial modifications of fruits after irradiation.

## 4. CONCLUSIONS

E-beam processing was effective to prevent *Guignardia citricarpa* development in culture. A low radiation dose of 2.5kGy was enough to inhibit fungal growth and doses higher than 5.0kGy completely inactivate *G. citricarpa*. Next approaches include E-beam processing to naturally infected oranges to evaluate radio susceptibility of fungi on fruits in order to validate these findings to a commercial application of radiation treatment to fruits itself.


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