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Gamma Radiation in the Control of Decay in Strawberries, Grapes, and Apples

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SUMMARY

Radio-sensitivity of Penicilium expansum and Botrytis cinerea conidia was proportional to the gamma dose and dependent on the nature of the suspending medium. In addition, for B. cinerea, recovery was modified by the holding period and the nature of the suspending medium following irradiation. Irradiation at from 100,000 to 300,000 rep (in rad, 91,280 to 273,840) substantially reduced gray mold and Rhizopus rot of strawberries during storage at 75°F for 3 days or at 45°F for 10 days. Doses of 300,000 rep prevented decay of berries stored a total of 8 days at alternating temperatures of 41°F and 75°F; injury in fruit irradiated at this dose was visible after 4 days' storage and severe injury was observed after 8 days. Under these conditions, doses of 200,000 rep prevented decay for 7 days, and 100,000 rep for 6 days without visible injury to berries. At 75°F a dose of 50,000 rep did not reduce growth of P. expansum in Jonathan apples regardless of the age of infection. A dose of 100,000 rep significantly reduced young infections (1-day-old) while 200,000 rep was required to check decay in 4-day-old infections. Doses exceeding 200,000 rep injured the fruit. Tokay grapes inoculated with B. cinerea regained free of decay for 4 days at 75°F after at least 500,000 rep; to prevent decay for 10 days' storage, a dose of 500,000 rep was required. Time of appearance and intensity of rotting were inversely proportional to the dose.

Studies on strawberries, grapes, and apples reported in this paper are part of an extensive investigation to attempt control of postharvest diseases with gamma radiation. Experiments were designed to determine whether disease control was possible within a dose range that would not injure fruit. Two previous papers have dealt with oranges and lemons, and peaches (2, 3). These studies have shown that although a relatively low dose of radiation can cause marked injury, somewhat lower doses can effectively delay fungal growth and spoilage. Simi-
RADIATION IN THE CONTROL OF DECAY IN FRUIT

Larly; radiation together with refrigeration, which can delay radiation injury, as well as retard decay, may combine to prolong the shelf life of fruit (2). Recently Nelson et al. (5) studied the influence of beta radiation (electron source) on the appearance of Tokay and Thompson seedless grapes and strawberries held at 3-4°C.

The organisms and fruits investigated differ in their reactions to gamma radiation. Therefore, the results are dependent on the combined influence of the susceptible-parasite sensitivities. The approaches followed in this study were: (a) effect of radiation on the fungus alone, (b) exploration of some factors responsible for survival of the organisms in association with the living susceptible, (c) use of refrigeration as an adjunct to radiation and (d) effect of radiation on texture and color of the fruit at various doses. A preliminary report of these investigations has been made (1).

MATERIALS AND METHODS

The methods used to prepare conidia of Penicillium expansum (I.K.) Thorn, and Botrytis cinerea Pers. ex Fr., and to determine their response to radiation were the same as those described for Penicillium digitatum Sacc. (2). Since both Tochinai’s and Czapek’s media were found to yield nearly 100% germination of unirradiated spores, comparisons were made of the influence of these media on total germination following irradiation.

Twelve strawberries in perforated polyethylene bags were included in each of 16 sealed cans to receive a selected dose. Since a high percentage of strawberries available on the Chicago market are always contaminated with B. cinerea and Rhizopus stolonifer (Ehr. ex Fr.) Vaink, artificial inoculations were unnecessary. After irradiation, equal numbers of these strawberries were held at 75°F for 3 days or at 41°F for 10 days. In another experiment, samples were held at 75°F for 2 days, then placed in the refrigerator (41°F) for 4 days, returned to a temperature of 75°F, and observed immediately and twice daily for an additional 2 days. Controls for each experiment consisted of unirradiated, identically prepared material. Tissue was examined microscopically to determine presence of mycelium in berries which had not shown visible signs of an infection. Records were also made of any changes in appearance of the berries as a result of radiation.

In order to insure a high incidence of gray mold, disease-free Tokay grapes were inoculated by dipping into a dense suspension of B. cinerea spores. After a 24-hr incubation period in a humidity chamber, small bunches of the inoculated grapes (about 20 grapes) were placed in perforated polyethylene bags in No 2 cans. Eight sealed cans of inoculated grapes received each selected dose. Records were made when inoculated unirradiated controls showed infection (within 4 days at 75°F) and periodically for an additional 6 days. In all trials, replicates of the unirradiated inoculated controls were infected.

Selected Jonathan apples, which had been in storage at 35°F, were surface disinfected with 1-1000 HgCl and rinsed several times in tap water. Approximately 15 punctures were made at the calyx end and on one cheek of each apple to provide infection courts. Inoculations were made by dipping punctured fruit into a conidial suspension for 15 seconds. Counts of viable spores in the inoculating medium averaged 40,000 spores/ml. Controls were similarly prepared and either inoculated with the fungus or dipped into sterile distilled water. Following inoculation, the apples were held in humidity chambers at 75°F for either 1 day or 4 days, prior to irradiation. All apples, regardless of the incubation period, were irradiated at the same time. Two apples were packed in a perforated polyethylene bag and placed in a No 2 can. Eight apples of each designated incubation period were irradiated at a selected dose at each trial. All inoculated unirradiated control samples produced a uniform infection when incubated at 75°F. Infections were scored on the extent of development of the rotted areas surrounding points of inoculation. All apples were scored 6 and 10 days following irradiation when control samples showed maximum rot as defined by the infection rating. The infection rating used is as follows:

**Rating** Description
0 No rot around point of puncture.
1 Rotted area discrete, slow in enlarging, no coalescence of spots, no sporulation on surface.
2 Rotted spot enlarging, little or no coalescence of spots, no sporulation on surface.
3 Less than 1/2 of apple rotted, spots beginning to coalesce, sporulation sparse to just beginning on surface.
4 1/2 to 3/4 of apple rotted, rotted spots coalesced, sporulation on surface. 
5 3/4 to 4/4 of apple rotted, spots coalesced and enlarged, profuse sporulation on surface.

Gamma radiation was provided from fission products in spent fuel elements and arranged in the High Level Gamma Irradiation Facility at the Argonne National Laboratory, Lemont, Illinois. Doses given in rep (to convert rep to rads, multiply rep by 91.28%) are approximate within 10%.

Studies on spores. The radio-sensitivity of spores differed in the two media. Tochinai’s medium provided protection and showed a higher percentage of germination of the conidia than Czapek’s medium over the range of doses tested from 50,000-250,000 rep. Under the conditions of the experiment a dose between 100,000 and 500,000 rep would be recorded to prevent germination of P. expansum conidia in either medium (Figure 1). From earlier studies (2) doses of from 500,000 to 1,000,000 rep prevented germination of R. stolonifer spores.

Previous experiments indicated that there was protection, recovery, and an increase in germination from prolonging the period irradiated spores were kept in a suspending medium before they were plated out. To test this observation, conidia of B. cinerea were suspended in distilled water and in Tochinai’s and Czapek’s media and kept at 38°F for 1, 2, and 6 days after irradiation (Table 1). The procedure to determine the percentage germination after a 20-hour incubation at room temperature was the same as for Penicillium digitatum (2).

Rate of recovery and protection afforded conidia from the suspending medium were not consistently better in Czapek’s

![Figure 1. Germination of gamma-irradiated conidia of Penicillium expansum in Tochinai’s and Czapek’s media.](image-url)
solution than in distilled water alone. However, rate of recovery was higher in Tochimai's medium than in either distilled water or Czapek's medium. Hence germination of irradiated conidia may be dependent on factors such as the type of medium as well as the length of time spores are held after irradiation before they are plated out. A dose of from 500,000 to 1,000,000 rep was required to prevent germination of B. cinerea conidia in Tochimai's medium for 1 day following irradiation; whereas, to prevent germination for 2 to 6 days after irradiation, treatment with 1,000,000 to 2,000,000 rep was necessary.

Studies with fruit

(1) Effect of gamma radiation and length of refrigeration on gray mold and Rhizopus rot of strawberries. Within the range of doses tested, i.e., 100,000 to 300,000 rep, there was an appreciable reduction in decay resulting from both E. stolonifer and B. cinerea after 3 days’ storage at 75° F (Figure 2) or after 10 days’ storage at 41° F (Figure 3). The variability in the initial concentration of inoculum of B. cinerea and E. stolonifer in these berries before irradiation made a direct comparison impossible of the relative survival of these fungi. Following 2 days’ storage at 75° F and an additional 4 days at 41° F, no rot had developed at any of the doses tested, while the unirradiated controls were almost completely rotten (Table 2). At 1,000,000, 750,000, and 500,000 rep, adverse effects of irradiation were noted after 2 days at 75° F, and at 300,000 rep only after an additional 4 days at 41° F. Injury to the fruit after irradiation occurred in the following order: softening, leaking, and finally bleaching of color. These symptoms appeared sooner and more intensely in proportion to the irradiation dose. Additional periods of storage following refrigeration (41° F) showed that the mycelium was present and still viable, since rot developed at 100,000 rep and finally at 200,000 rep (Table 2). With strawberries, apparently a delicate balance exists between those doses causing visible injury and those required to retard the development of the pathogen for a prolonged period of time.

(2) Effect of gamma radiation and storage time on development of gray mold rot of grapes. As determined visually.

TABLE 1

Per cent germination of Botrytis cinerea conidia in different suspending media at three intervals following irradiation at various levels

<table>
<thead>
<tr>
<th>Days after irradiation at 38° F</th>
<th>Dose (rep)</th>
<th>Suspending medium</th>
<th>Tochimai</th>
<th>Czapek</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (control)</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>100,000</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>2</td>
<td>175,000</td>
<td>100</td>
<td>75.3</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>300,000</td>
<td>100</td>
<td>69.3</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>4</td>
<td>500,000</td>
<td>100</td>
<td>77.6</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>5</td>
<td>1,000,000</td>
<td>100</td>
<td>71.6</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>6</td>
<td>2,000,000</td>
<td>100</td>
<td>71.6</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

1 Average percentage germination of 300 spores in each of 3 replicates.
2 To convert rep to rad, multiply rep by 91.28%.

Figure 2. Average percentage of irradiated strawberries infected by Botrytis cinerea (gray mold) and Rhizopus stolonifer (Rhizopus rot) after 3 days at 75° F.

TABLE 2

Effect of irradiation at various dose levels on the appearance of strawberries and the per cent infected fruit following alternate storage at 75° F and 41° F

<table>
<thead>
<tr>
<th>Approx. dose (rep)</th>
<th>Storage temperature and time intervals after irradiation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 days at 75° F</td>
</tr>
<tr>
<td>0 (control)</td>
<td>88</td>
</tr>
<tr>
<td>100,000</td>
<td>0.0</td>
</tr>
<tr>
<td>200,000</td>
<td>0.0</td>
</tr>
<tr>
<td>500,000</td>
<td>0.0</td>
</tr>
<tr>
<td>750,000</td>
<td>0.0</td>
</tr>
<tr>
<td>1,000,000</td>
<td>0.0</td>
</tr>
</tbody>
</table>

1 Infections due to Rhizopus stolonifer and Botrytis cinerea.
TABLE 3
Effect of irradiation at various dose levels on the appearance of inoculated grapes and the per cent infected fruit following several storage periods at 75° F

<table>
<thead>
<tr>
<th>Dose (rep)</th>
<th>4 days</th>
<th>8 days</th>
<th>10 days</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Appearance</td>
<td>% Infected</td>
<td>Appearance</td>
</tr>
<tr>
<td>0 (control)</td>
<td>Color bleached from rotted areas, grapes soft, surface mold</td>
<td>100</td>
<td>As after 4 days</td>
</tr>
<tr>
<td>50,000</td>
<td>Color bleached from rotted grapes, some area soft</td>
<td>100</td>
<td>As control</td>
</tr>
<tr>
<td>100,000</td>
<td>Color good on most grapes, rot started</td>
<td>66</td>
<td>As control</td>
</tr>
<tr>
<td>200,000</td>
<td>Color good, grapes firm</td>
<td>0.0</td>
<td>Color good on most grapes, rot started, some grapes soft</td>
</tr>
<tr>
<td>300,000</td>
<td>Color good, grapes firm</td>
<td>0.0</td>
<td>Color good, grapes firm, rot started</td>
</tr>
<tr>
<td>500,000</td>
<td>Color good, grapes firm</td>
<td>0.0</td>
<td>No odor, grapes firm, color good</td>
</tr>
<tr>
<td>1,000,000</td>
<td>Color good, grapes firm</td>
<td>0.0</td>
<td>Color good, off-odor, grapes firm</td>
</tr>
</tbody>
</table>

1 Infections due to Botrytis cinerea.

no color changes were apparent at any of the doses selected for the experiment (Table 3). Similarly, the grapes retained their firmness and, with the exception of a fermented off-odor of the grapes receiving 1,000,000 rep, no other detectable changes occurred in the fresh product over the range of the doses tested. At 1,000,000 and 500,000 rep, no rot developed in any of the replicates for a period of 10 days at 75° F, while unirradiated controls were completely rotted within 4 days. Within the range of doses tested between 50,000 and 300,000 rep, the first appearance and total amount of decay from Botrytis cinerea were inversely proportional to the dose.

(3) Influence of radiation and storage time on development of injury and blue mold rot of apples. At a dose of at least 100,000 rep, there was significant reduction in the development of rot after 6 days' storage at 75° F for those apples inoculated one day prior to irradiation (Table 4). However, after 10 days' storage at 75° F, the minimal dose required to significantly reduce the advancement of infection was 200,000 rep. A 4-day incubation period required a minimal dose of 100,000 rep to show significant decay reduction at the 6-day scoring; a minimum of 200,000 rep, and more significantly a dose of 300,000 rep, showed decay reduction at the 10-day scoring. At 50,000 rep, there was no delay in rotting of apples regardless of the incubation period prior to irradiation. Apparently, a minimal dose to retard blue mold on apples is the same whether an incubation period of 1 day or 4 days precedes irradiation.

Whole apples were injured by gamma radiation at doses of 300,000, 500,000, and 1,000,000 rep. Injury was in the form of skin and flesh browning and softening in proportion to the dose. No adverse effects were visible following doses of either 100,000 or 200,000 rep when apples were held 10 days at 75° F.

DISCUSSION

It is difficult, on the basis of our knowledge of the mechanism of the effect of radiation on susceptible and pathogenic cell constituents, to give an explanation for the delay in rotting of fruit as observed in our experiments. The delay in rotting of the fruits as a result
radiation in a non-sterilizing range of doses may be the result of (a) a partial inactivation and/or recovery of the pathogen, (b) a lengthening of the latent period of infection, or (c) changes in the susceptible's resistance to infection. Those doses which are high enough to eradicate the fungus also produce irreversible injury. While the action of radiation has been likened to that exhibited by a fungistat (5), the term “fungistat” is generally restricted to chemicals which can arrest development of fungi while in continuous contact.

The recovery of the pathogen from irradiation in the susceptible and on culture media is dependent on the time after irradiation and on the nature of the substrate. A metabolite which may be responsible for post-irradiation recovery has not been demonstrated. However, some components of proteinaceous materials can afford protection and stimulate recovery of bacteria (4, 7). Sulphydryl compounds, such as cysteine, for example, may compete with or replace sulphydryl groups of certain enzymes which are sensitive to gamma radiation (4). A fungal agent of decay in association with the substrate in the form of susceptible tissue or a complex organic culture medium may be protected by this reaction.

Lower doses of radiation are needed to retard young infections of apple (Table 4) after a 1-day incubation than to retard infection after a 4-day incubation. P. expansum may be more susceptible to radiation in its early than its later stage of growth. Sherman and Albus (6) demonstrated that during the lag growth phase of Escherichia coli, cells are more susceptible to both chemical and physical changes of environment than cells from the later portion of the logarithe and stationary growth phases. This sensitivity in “physiologie youth” has also been shown in our radiation work with Penicillium digitatum mycelium (2).

The use of radiation for fresh produce pasteurization remains an experimental tool, for it has not yet improved its safe, practical, or economical worth. However, it does merit attention in a study of pathogen radio-sensitivity and threshold levels of host injury. Finally, radiation offers an approach to understanding common physiological diseases, since it can often induce nearly the same disorders and types of symptoms expressed after produce has been aged, frozen, dried, or overheated.

LITERATURE CITED