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

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## SUMMARY

Radio-sensitivity of *Penicillium 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 41° 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* remained free of decay for 4 days at 75° F after at least 200,000 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 post-harvest 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-

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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.) Thom, 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 Tochinnai'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.) Vuillemin, 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 disinfested with 1–1000 HgCl<sub>2</sub> 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 1/3 of apple rotted, spots beginning to coalesce, sporulation sparse to just beginning on surface.
4	1/3 to 1/2 of apple rotted, rotted spots coalesced, sporulation on surface.
5	1/2 to 3/4 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%.

Selected doses for the different fruits in individual No. 2 cans were replicated a total of at least 8 times in 2 separate trials. Following irradiation, the lids of the cans were cut off and replaced loosely to permit ventilation. Examinations were made daily to trace the development of rot and any changes in the general appearance of the fruit.

### RESULTS

**Studies on spores.** The radio-sensitivity of spores differed in the two media. Tochinnai's medium provided protection and resulted in 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 300,000 and 500,000 rep would be required to prevent germination of *P. expansum* conidia in either medium (Figure 1). From earlier studies (3) 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 Tochinnai'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

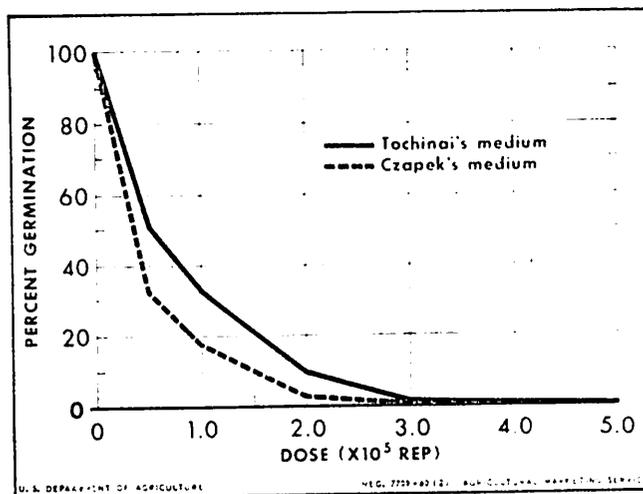


Figure 1. Germination of gamma-irradiated conidia of *Penicillium expansum* in Tochinnai's and Czapek's media.

solution than in distilled water alone. However, rate of recovery was higher in Tochinal'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 media 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 Tochinal'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 *R. 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 *R. 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 rotted (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<sup>1</sup> of *Botrytis cinerea* conidia in different suspending media at three intervals following irradiation at various levels

Days after irradiation at 38° F	Dose (rep) <sup>2</sup>	Suspending medium		
		Tochinal	Czapek	Water
1	0 (control)	100	100	100
2		100	100	100
6		100	100	100
1	100,000	100	100	87.3
2		100	100	76.5
6		100	100	100
1	175,000	100	47.8	58
2		100	100	69.8
6		100	100	100
1	300,000	100	18.3	33
2		100	69.3	63.4
6		100	77.6	79.8
1	500,000	72.0	3.1	1.6
2		84.3	2.6	13.5
6		82.8	71.6	46.3
1	1,000,000	0	0	0
2		5.5	0	0
6		59.5	0	2.7
1	2,000,000	0	0	0
2		0	0	0
6		0	0	0

<sup>1</sup> Average percentage germination of 300 spores in each of 3 replicates.

<sup>2</sup> To convert rep to rad, multiply rep by 91.28%.

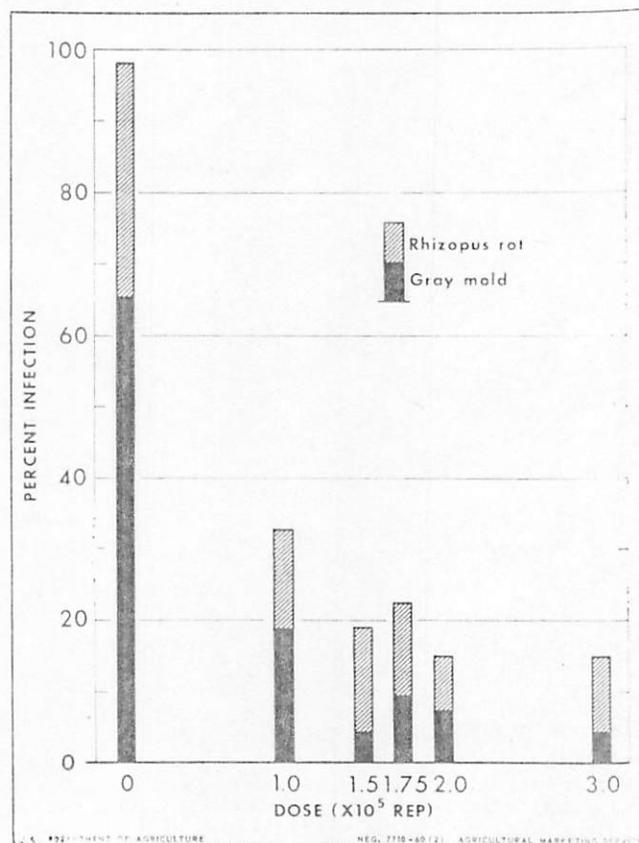


Figure 2. Average percentage of irradiated strawberries infected by *Botrytis cinerea* (gray mold) and *Rhizopus stolonifer* (Rhizopus rot) after 3 days at 75° F.

TABLE 2

Effect of irradiation at various dose levels on the appearance of strawberries and the per cent infected<sup>1</sup> fruit following alternate storage at 75° F and 41° F

Approx. dose (rep)	Storage temperature and time intervals after irradiation							
	2 days at 75° F	% Infected	Additional 4 days at 41° F	% Infected	Additional 1 day at 75° F	% Infected	Additional 1 day at 75° F	% Infected
0 (control)	Surface mold; rotted, soft	88	Surface mold; rotted, soft	100	Surface mold; rotted, soft	100	Surface mold; rotted, soft	100
100,000	Firm, color good	0.0	Firm, no color change	0.0	Surface mold, soft	77	Surface mold; soft from rot	100
200,000	Firm, color good	0.0	Firm, no color change	0.0	Firm, no color change	0.0	Surface mold; soft from rot	84
300,000	Firm, no color change	0.0	Slightly soft, no color change	0.0	Very soft, some leaking, color bleached	0.0	Very soft, leaking, some color bleached	0.0
500,000	Very slightly soft, no color change	0.0	Very soft, leaking, color bleached	0.0	Very soft, leaking, color bleached	0.0	Very soft, leaking, color bleached	0.0
750,000	Soft leaking, no color change	0.0	Very soft, leaking, color bleached	0.0	Very soft, leaking, color bleached	0.0	Very soft, leaking, color bleached	0.0
1,000,000	Very soft; leaking; no color change	0.0	Very soft, leaking, color bleached	0.0	Very soft, color bleached	0.0	Very soft, color bleached	0.0

<sup>1</sup> 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<sup>1</sup> fruit following several storage periods at 75° F

Dose (rep)	4 days		8 days		10 days	
	Appearance	% Infected	Appearance	% Infected	Appearance	% Infected
0 (control)	Color bleached from rotted areas, grapes soft, surface mold	100	As after 4 days	100	As after 4 days	100
50,000	Color bleached from rotted grapes, some areas soft	100	As control	100	As control	100
100,000	Color good on most grapes, rot started	66	As control	100	As control	100
200,000	Color good, grapes firm	0.0	Color good on most grapes, rot started, some grapes soft	54	As control	100
300,000	Color good, grapes firm	0.0	Color good, grapes firm, rot started	20	Color good on most grapes, surface mold	57
500,000	Color good, grapes firm	0.0	No odor, grapes firm, color good	0.0	Color good, no odor, grapes firm	0.0
1,000,000	Color good, grapes firm	0.0	Color good, off-odor, grapes firm	0.0	Color good, off-odor, grapes firm	0.0

<sup>1</sup> 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 those 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 *B. 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

TABLE 4

Average infection ratings of whole apples inoculated with *Penicillium expansum*, incubated for 1 or 4 days prior to irradiation with various doses, and then held up to 10 days at 75° F

Approx. dose (rep)	Incubation period prior to irradiation					
	1 day incubation			4 days incubation		
	Initial	After 6 days	After 10 days	Initial	After 6 days	After 10 days
0 (non-inoculated control)						
0 (inoculated control)	1.2	4.6	5.0	2.3	5.0	5.0
50,000		4.3	5.0		4.6	5.0
100,000		2.2	3.3		3.2	4.7
200,000		1.3	2.2		3.0	3.2
300,000		1.0	1.5		2.0	2.6
500,000		1.0	1.2		2.1	2.3
1,000,000		1.1	1.3		1.9	2.0
Least significant difference @ 19:1		1.7	1.9		1.6	1.8

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 pathogen 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

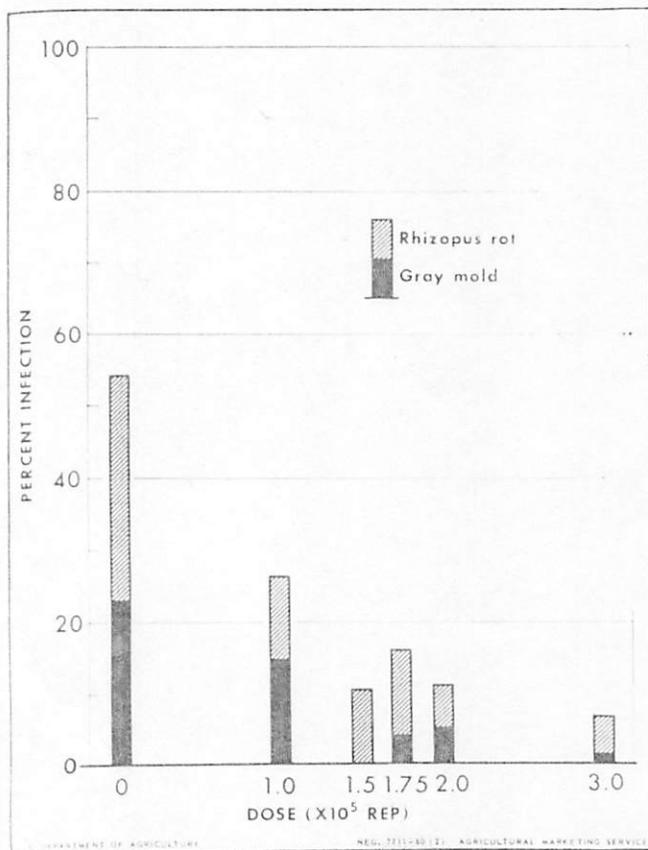


Figure 3. Average percentage of irradiated strawberries infected by *Botrytis cinerea* (gray mold) and *Rhizopus stolonifer* (Rhizopus rot) after 10 days at 41° F.

of 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). Sulfhydryl compounds, such as cysteine, for example, may compete with or replace sulfhydryl 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 logarithmic and stationary growth phases. This sensitivity in "physiologic 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 proved 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.

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