Influence of Gamma Radiation on the Microflora of Cucumber Fruit and Blossoms[1-3]

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Numerous and extensive investigations have been conducted on the influence of ionizing radiations on pure cultures of microorganisms. Various aspects of such research have been subject of a rather comprehensive review by Rayman and Byrne (1957). In contrast to pure culture studies, the present report deals with the influence of gamma radiation on several of the microbial groups present in the heterogeneous population that occurs naturally on freshly harvested cucumber fruit and blossoms.

MATERIALS AND METHODS

Collection of samples. Immature cucumber fruit, consisting of no. 1 size Model variety (¾ to ⅞ in. diameter), in 1 to 2 bushel amounts was obtained on the 7 sampling dates indicated (table 1) from a cucumber receiving and grading station located at Zebulon, North Carolina, about 20 miles from the laboratory. Individual samples, prepared at each sampling date, represented 10 cucumbers each; these were placed in polyethylene-cellophane laminated bags and heat sealed. Cucumber samples were collected and handled using aseptic precautions. The 68 individual bag samples of cucumbers (10 per bag) averaged 123 g each, with an average unit (per cucumber) weight of 12.4 g. Fruit samples were collected, bagged, and refrigerated at 4 C during the afternoon of each sampling date; irradiation treatments were started the following morning.

Partially dried cucumber blossoms were either removed by sterile tweezers from no. 1 size fruit or obtained, using aseptic technique, from under the cucumber grading machine. Blossom samples were collected twice during the season (table 1) and handled according to the same general procedure described for cucumber fruit. The 6 samples collected and prepared June 10 consisted of 100 blossoms each; the 10 samples collected June 17 represented 25 blossoms each. The average sample weight for the June 10 and 17 collections was 2.75 g (100 blossoms) and 0.69 g (25 blossoms), respectively. The average unit (per blossom) weight was the same for samples collected on both dates, namely 0.0275 g. Blossom samples, after counting, weighing, and bagging, were stored in the refrigerator until irradiated.

Irradiation of samples. The samples of cucumber fruit and blossoms, in sealed polyethylene bags, were irradiated at 22 to 25 C and 65 per cent relative humidity in a fixed cobalt-60 gamma source of 2300 curies capacity with a previously calibrated dose rate of 0.2 megarep per hr. Samples receiving dosages from 0.05 to 2.0 megareps (15 min to 10 hr exposure time) were irradiated during the day, refrigerated overnight, and prepared for bacteriological tests the following morning. Samples given the 3.0 megarep dose, requiring 15 hr

TABLE 1

Information on samples of cucumber material collected for gamma radiation studies

Cucumber Material Sampled	Samplings During 1959 Season	Number of Samples	Radiation Dosages Used	
	date		*no.*	*range in megareps*
Fruit	6/18	6	6	0*-3.0
	6/22	14	7†	0-1.0
	6/24	8	7	0-1.0
	6/29	9	8	0-1.5
	7/1	9	8	0-1.5
	7/6	11	9‡	0-3.0
	7/9	11	9‡	0-3.0
Blossoms	6/10	6	6	0-3.0
	6/17	10	9	0-3.0

* Control samples usually run in duplicate for each radiation experiment during the season.

† Samples run in duplicate at each dosage.

‡ Samples run in duplicate at the 0.10 megarep level.

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exposure, were finished at night and included with the other samples the next day. The radiation facility used was located in the School of Textiles, North Carolina State College, and consisted of a Gammacell-220[5] unit.

Preparation of samples. Cucumber fruit and blossom samples were prepared for bacteriological examination as described in detail by Etchells *et al.* (1958). Briefly, the procedure was as follows: previously weighed blossom samples were removed from the sealed polyethylene bags using aseptic technique, placed in sterilized, stainless steel Omnimixer[6] blending chambers together with sufficient 0.85 per cent saline to make a final dilution of 1:10 or 1:50 depending on the sample weight. The mixture was then blended for 1 min at 14,000 rpm and decimal dilutions made as desired. The weighed fruit samples were removed from the sealed bags as described for blossoms and, together with an equal weight of saline in 1-L flasks, were shaken by hand 100 times; decimal dilutions were then prepared from the washings.

Cultural procedure. Population estimates were obtained for several groups of microorganisms occurring on irradiated and control samples of cucumber fruit and blossoms by use of selective media and employing plating, streaking and deep agar shake culture techniques, most of which have been described in detail by other workers. Decimal dilutions of the samples were examined for eight microbial groups as outlined briefly below.

(1) *Total aerobes* were determined by streaking with a calibrated platinum loop (0.01 g capacity) onto previously poured plates of glycerol asparaginate agar (Conn, 1921). (2) *Aerobic spores* present in heated samples (80 C for 10 min) were enumerated by plating with nutrient agar (Difco).[7] (3) *Total anaerobes* were estimated by culturing in the basal yeast extract starch bicarbonate (YESB) agar of Wynne, Schmieding, and Daye (1955) using the modified deep agar shake tubes of Miller, Garrett, and Prickett (1939). (4) *Anaerobic spores* present in heated samples (80 C for 10 min) were cultured as described for total anaerobes. (5) *Coliform bacteria* were determined by the plating or streaking techniques using brilliant green bile agar (Difco). (6) *Acid-forming bacteria* were estimated by plating with an experimental selective medium, using a trypticase sugar agar base,[8] plus 0.01 per cent brom cresol green dye (Difco), 0.5 per cent yeast extract (Difco), 200 ppm Actidione,[9] and 15 ppm polymyxin B.[10] The basal medium with added dye and yeast extract was adjusted to pH 5.7 to 5.8 at the time of preparation. The two antibiotics were added aseptically to the previously melted and cooled agar, just prior to plating. This medium was designed in an effort to separate relatively low populations of lactic acid bacteria occurring on cucumber material from rather high populations of other microbial groups that might be present, chiefly the mesophilic aerobes. (7) *Yeasts* and (8) *Molds* (filamentous fungi) were determined by streaking onto plates of nitrogen base agar prepared from Wickerham's (1951) yeast nitrogen base broth (Difco) as described by Etchells *et al.* (1953, 1958). This medium was modified for the present study by the addition of 0.01 per cent yeast extract and acidification with 1.6 ml of sterile 5.0 per cent tartaric acid (instead of 3 ml) per 100 ml of melted agar. The modified medium (pH 3.5) inhibited the bacteria, yet permitted good growth of yeasts together with adequate but restricted colonial development of molds.

Measurement of cucumber firmness. The USDA Fruit Pressure Tester, devised by Magness and Taylor (1925) was used to measure cucumber firmness according to the procedure described by Bell, Etchells, and Jones (1955). In the present study, each pressure test value represents the average results for 10, no. 1 size cucumbers, each receiving a single center punch and recorded to the nearest pound resistance to the $^5/_{16}$ in. tip of the tester.

RESULTS

Initial Population Studies

Rather high populations of a heterogeneous microflora were found to occur on cucumber fruit and blossoms (table 2). Consistently higher numbers of all eight types of organisms, calculated on either a weight or unit basis, were obtained from blossom samples as compared to fruit. Bacteria which grow aerobically predominated on both types of cucumber material and the coliform group comprised about 25 to 30 per cent of this population. The aerobic and anaerobic spores accounted for less than 0.5 per cent of the corresponding bacterial flora that grew under each condition. The populations of acid-forming bacteria reported (in table 2) represent our first degree of success, among numerous efforts, to separate this important group of organisms from the exceedingly high populations of other microbial groups usually present on samples of cucumber material. Within certain limitations, the selective medium used in the present study is believed to be fairly satisfactory; however, a continued effort is being made to improve it.

[5] Atomic Energy of Canada Ltd., Ottawa, Canada. It is not the policy of any of the organizations represented by the authors to recommend the products of any company over similar products of any other company. The name is supplied for informational purposes.

[6] Ivan Sorvall, Inc., Norwalk, Connecticut.

[7] Difco Laboratories, Inc., Detroit, Michigan.

[8] Baltimore Biological Laboratory, Inc., Baltimore, Maryland.

[9] The Upjohn Company, Kalamazoo, Michigan.

[10] Burroughs-Wellcome and Company, Tuckahoe, New York.

Initial populations of the microbial groups present on cucumber fruit and blossoms are generally influenced by several factors such as the prevailing growing conditions, the production area sampled, and the period of harvest during which samples are collected. However, the population ratios existing among the various microbial groups were relatively constant for the samples used in the present study.

Radiation Studies

The survival curves for eight microbial groups occurring on cucumber fruit and blossoms exposed to gamma rays are shown in figures 1 and 2. The values plotted represent the seasonal average for seven experiments.

Fruit. The results indicate that of the microbial groups shown in figure 1, the nonsporeforming bacteria were the most sensitive to gamma radiation. Dosages of 0.5 to 0.75 megarep reduced the total aerobic and anaerobic bacterial populations to levels equivalent to spore counts and eliminated the coliform and acid-forming bacteria from the samples. Bacterial spores were observed to be the most resistant to radiation; anaerobic and aerobic spore forms were recovered from the fruit after dosages of 1.0 and 2.0 megareps, respectively. The aerobic bacilli were present in much higher populations initially than the anaerobic bacilli; therefore, the difference in response to radiation does not necessarily mean a higher resistance demonstrated for aerobic spores.

The yeasts and molds appeared to be considerably more resistant than vegetative cells of bacteria. Viable cells of both of these groups of fungi were recovered from fruit exposed to 1.0 megarep even though the initial populations demonstrated they were present in substantially lower numbers than most of the bacteria.

No organism was cultured from fruit samples receiving a dose of 3.0 megareps.

Blossoms. The microbial destruction picture for samples of irradiated cucumber blossoms (figure 2) was essentially the same as that described for fruit. The initial populations of the eight microbial groups were higher on blossoms as compared to fruit but the corresponding microbial survival curves parallel one another for the irradiation of both types of sample material. The yeasts and molds again appeared to be second only to bacterial spores in radiation resistance. No organisms except bacterial spores were recovered from blossom samples irradiated at the 1.5 megarep level and no spores were cultured from samples receiving 3.0 megareps. Because of the amount of particulate material in the primary dilutions of blended blossom samples it was not possible to make population estimates below 1×10^2. Thus, the data do not necessarily mean that sterility was attained even at the highest radiation dosage used (3.0 megareps).

TABLE 2

Microbial populations on cucumber fruit and blossoms (1959 season)

Microbial Group	Colony Counts*			
	Cucumber fruit		Cucumber blossoms	
	Per g	Per unit†	Per g	Per unit
	thousands	*thousands*	*thousands*	*thousands*
BACTERIA				
Aerobes				
Total	15,950.0	182,320.0	18,200,000	476,000
Spores	16.7	218.0	67,800	1,940
Anaerobes				
Total	1,830.0	19,800.0	3,092,000	78,760
Spores	0.8	9.8	2,100	191
Coliforms	3,940.0	49,125.0	6,400,000	167,530
Acid-formers	4.9	60.0	26,000	765
YEASTS	1.6	18.0	3,030	82
MOLDS	3.4	44.0	11,300	295

* Counts shown represent the average for seven samplings of no. 1 size Model variety cucumbers and two samplings of blossoms collected during the 1959 season.

† Average unit weights: cucumber, 12.4 g; cucumber blossom, 0.0275 g.

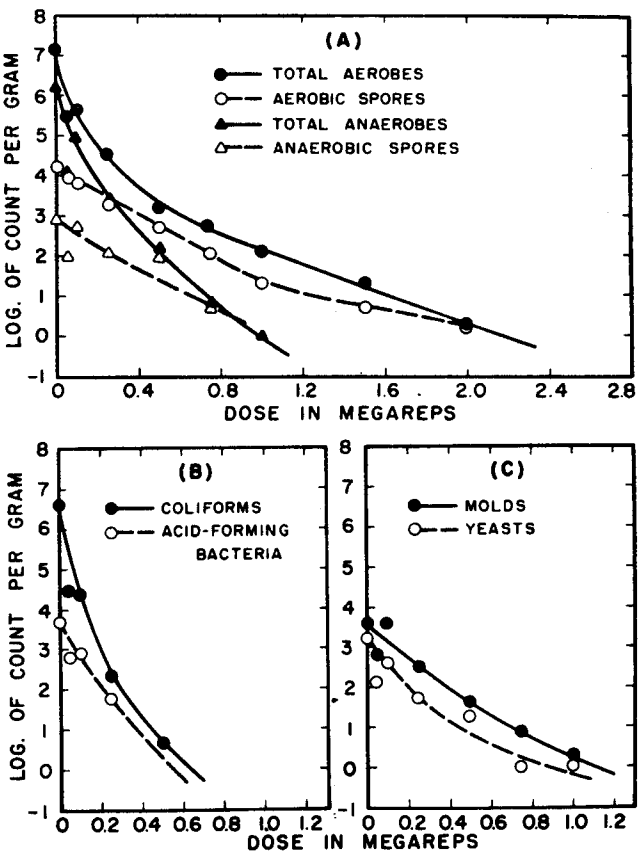

Figure 1. Influence of radiation on populations of certain microbial groups on cucumber fruit. *Part A*, counts less than 1 per g beyond 2.0 megareps; *part B*, counts less than 1 beyond 0.5 megarep; and, *part C*, counts less than 1 beyond 1.0 megarep.

Firmness of cucumbers. The influence of radiation on the firmness of no. 1 size Model variety pickling cucumbers is presented in figure 3. The values plotted represent the mean for seven experiments. Loss in cucumber firmness was found to be in direct relationship to the dose used. Increasingly higher doses of radiation resulted in correspondingly lower cucumber firmness values as compared to nonirradiated controls. Cucumbers subjected to 3.0 megareps, the highest level used, lost approximately 60 per cent of their initial firmness. The decrease in texture observed for irradiated cucumbers is similar to that reported earlier by Kertesz (1956) for fresh beets and carrots.

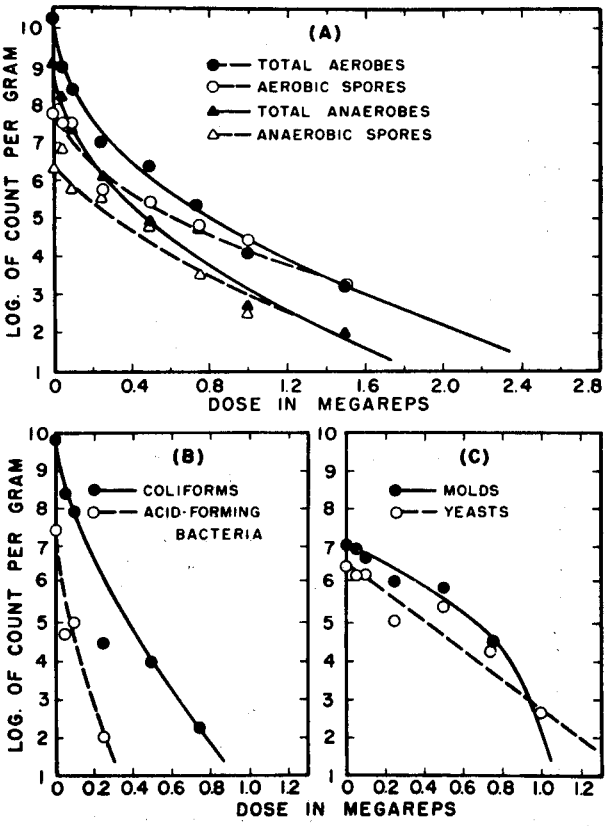

Figure 2. Influence of radiation on populations of certain microbial groups on cucumber blossoms. *Part A*, counts less than 100 per g beyond 1.5 megareps; *part B*, counts less than 100 beyond 0.75 megarep; and, *part C*, counts less than 100 beyond 1.0 megarep.

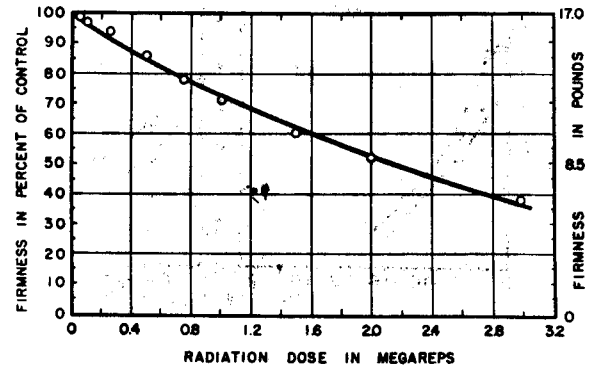

Figure 3. Influence of radiation on the firmness of cucumber fruit.

Discussion

In the present study, the microorganisms subjected to radiation represented a heterogeneous population occurring naturally on the plant material investigated. Expected differences might be anticipated as to radiation resistance for individual microbial groups from a natural source as compared to those where pure cultures of such groups were employed. However, the survival patterns for the microbial types irradiated herein are in general agreement with those observed by others when pure cultures were studied.

More specifically, Pepper, Buffa, and Chandler (1956) reported no difference in the resistance between aerobic spores as a group and anaerobic spores as a group when subjected to cathode rays. Essentially the same results were obtained in the present study where the destruction rates for the aerobic and anaerobic spores from a heterogeneous flora paralled one another and approached a logarithmic order.

Bridges, Olivo, and Chandler (1956) and Koh, Morehouse, and Chandler (1956) observed that cultures of yeasts and molds were more resistant to cathode rays than cultures of nonsporeforming bacteria. The current findings demonstrate a similar relationship with a heterogeneous flora. However, we were unable to observe the higher radiation resistance for yeasts as a group as compared to molds reported by Bridges *et al.* (1956). They tested 12 species in 6 genera of yeasts and 10 species in 2 genera of molds. Previous taxonomic studies, on cucumber material from the same production area in North Carolina as used in the current investigation, would indicate that species of at least 8 genera of yeasts and 34 genera of molds might be expected in the samples used herein (Etchells *et al.*, 1953, 1958; and Raymond *et al.*, 1959).

Lack of agreement in the present study with that of Bridges *et al.* (1956) with respect to the comparative resistance of yeasts and molds to radiation may be partly due to the species composition of the heterogeneous mold flora in our samples. In this connection, Webb, Thiers, and Richardson (1959) reported that *Alternaria* sp., *Aspergillus niger*, and *Aspergillus terreus* were eliminated from ground corn containing 23 per cent moisture with 0.35 megarads of gamma radiation (1 megarad = 1.08 megareps); however, single species of *Homodendrum*, *Penicillium*, and *Verticillium* survived a dose of 0.75 megarad at the same moisture level.

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Summary

Results of a study on the influence of gamma radiation on several microbial groups in the heterogeneous population occurring naturally on cucumber fruit and blossoms are presented. The initial populations of the different microbial groups were much higher on blossoms as compared to fruit but the corresponding microbial survival curves indicated that the destruction rates were comparable for the irradiation of both types of material.

Of the various microbial groups studied, the asporogenous bacteria, as represented by the coliform and acid-forming bacteria, were found to be the most sensitive to radiation; the aerobic and anaerobic spore forms were observed to be the most resistant. The yeasts and molds (filamentous fungi) appeared to be second only to bacterial spores in radiation resistance. However, the resistance of yeasts as a group and molds as a group was considered to be essentially the same. Furthermore, the studies indicate that, in general, the individual microbial groups in a heterogeneous natural flora occurring on cucumber material responded to radiation in a manner similar to that reported by other workers using pure cultures from comparable groups of microorganisms.

Increasingly higher doses of gamma radiation resulted in correspondingly lower cucumber firmness values as compared to nonirradiated controls.

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