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Effects of

OZONE ATMOSPHERES ON SPOILAGE OF FRUITS AND VEGETABLES

After Harvest



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Effects of OZONE ATMOSPHERES ON SPOILAGE OF FRUITS AND VEGETABLES

After Harvest

By DONALD H. SPALDING, *research plant pathologist, Market Quality Research Division, Agricultural Research Service*

SUMMARY

Effectiveness of ozone for reducing post-harvest decay of fruits and vegetables was tested at 0.5 p.p.m. ozone and 90 percent relative humidity.

Ozone did not reduce rhizopus and brown rot of peaches or rhizopus and botrytis rot of strawberries stored for 1 week at 35° F. Ozone treatment during storage had no influence after storage on the shelf life of peaches held in air for 4 days at 70°. Size of fungal nests was somewhat reduced in peaches and strawberries held in ozone at 60°, but nesting can be more effectively controlled by low temperature storage.

Present results confirmed previously reported data showing that 0.5 p.p.m. ozone did not injure peaches, but it damaged the caps of strawberries and caused the caps to dry and shrivel.

Ozone did not reduce rot in blueberries held

in ozone at 35° F. for 2 days followed by 4 days in air at 70°. Blueberries held in ozone for 6 days at 60° had essentially the same amount of rot as fruit held in air under otherwise identical conditions.

Cantaloups developed the same amount of rot regardless of the presence or absence of ozone when held for 7 days at 45° F. followed by 5 days at 60°.

Ozone inhibited surface mold growth without reducing gray-mold rot on Thompson seedless and Tokay grapes held for 6 or 7 days at 60° F.

The amount of alternaria rot developing in green beans held in ozone for 7 days at 45° F. and in air for 5 days at 60° did not differ significantly from that in untreated beans. Ozone injured exposed layers of beans during storage at 45°.

REVIEW OF PROBLEM

The U.S. Department of Agriculture has received numerous requests for information concerning the effectiveness of ozone for preventing spoilage of fruits and vegetables during storage. Pertinent literature covering the use of ozone in storage rooms was reviewed in an earlier publication.¹

Conflicting reports have been made of the effectiveness of ozone in reducing postharvest decay. Spalding² reported that ozone did not

reduce spoilage of strawberries or peaches held at 60° F. and 90 percent relative humidity, but did inhibit the surface growth of fungi and did eliminate the typical nesting appearance. Ridley and Sims,³ on the other hand, concluded that ozone reduced disease development in strawberries and peaches held at 35°, although their results with strawberries were not statistically significant.

The present study represents an extension of earlier work and considers the effect of ozone treatment during storage at different temperatures on storage life and subsequent shelf life of fruits or vegetables.

¹ SPALDING, D. H. APPEARANCE AND DECAY OF STRAWBERRIES, PEACHES, AND LETTUCE TREATED WITH OZONE. U.S. Dept. Agr. Mktg. Res. Rpt. 756, 11 pp., illus. 1966.

² SPALDING, D. H. EFFECT OF OZONE ON APPEARANCE AND DECAY OF STRAWBERRIES, PEACHES, AND LETTUCE. (Abstract.) *Phytopathology* 56: 586. 1966.

See also footnote 1.

³ RIDLEY, J. D., and SIMS, E. T., JR. PRELIMINARY INVESTIGATIONS ON THE USE OF OZONE TO EXTEND THE SHELF-LIFE AND MAINTAIN THE MARKET QUALITY OF PEACHES AND STRAWBERRIES. S.C. Agr. Expt. Sta. (Clemson) Res. Ser. 70: 1-22. 1966.

MATERIALS AND METHODS

Storage tests were made in rooms (13 by 8 by 9 feet) with relative humidity (RH) maintained at 90 percent with the aid of humidity sensing elements linked with a relay system to solenoid valves operating a spraying device. Humidity and temperature readings were recorded with a hygrothermograph.

One room was equipped with an ozone generator with a capacity of 40 grams per hour. The generator was located on a wall midway between the front and rear of the room, but was operated through a variable transformer placed outside the room. The concentration of ozone in the storage rooms was maintained at 0.5 p.p.m. in all studies.

Air samples were withdrawn at frequent intervals by using a portable pressure-vacuum air pump. The collection and analytical procedure was described in a previous report.⁴ The air was sampled through glass tubing (4 millimeters inside diameter) running from the outside of the chamber to the center of the wall opposite the location of the ozone generator.

The fruits and vegetables used were purchased at the wholesale market in Washington, D.C., with the exception of locally grown strawberries. Rooms were never loaded beyond 50 percent of capacity. The following tests were run.

Peaches

Test I.—Thirty-six hundred peaches were selected and placed at random in 4-qt. tills and inoculated with spores of the brown rot fungus (*Monilinia fructicola* (Wint.) Honey) or spores of the rhizopus rot fungus (*Rhizopus stolonifer* (Ehr. ex Fr.) Lind) by dipping them in beef extract-peptone broth suspensions of the fungal spores as previously described.⁵ The peaches were held at 70° F. for 24 hours to allow the spores to germinate and were then placed in the air and ozone rooms at 35° for 7 days, at which time the peaches were examined for rot. The peaches were then held in air at 70° and 90 percent RH for 2 and 4 days to determine shelf life, primarily by observations on the amount of rot.

Test II.—Twelve hundred peaches were selected and placed at random in 4-qt. tills. A peach with either brown rot or rhizopus rot was placed in the center of each basket to serve as a center for the development of a fungal nest. The peaches used as nesting centers were fully rotted, with the skin completely covered with

spore-bearing mycelia. Half of the test baskets were held with ozone and half without for 7 days at 60° F. and 90 percent RH. The peaches were then examined to determine the number of peaches in the fungal nest.

Strawberries

Test I.—One hundred and twenty pints of freshly harvested strawberries were selected and placed at random in polyethylene baskets. One-third of the baskets of strawberries were used to study *Botrytis* nesting, another third for *Rhizopus* nesting, and the remaining third were left undisturbed. A completely molded berry covered with sporulating mycelia of *Botrytis cinerea* (Pers. ex Fr.) or *Rhizopus stolonifer* was placed in the center of the appropriate basket. Half of the berries were then held for 7 days at 35° F. and 90 percent RH with 0.5 p.p.m. of ozone and half without ozone. Berries in half of the baskets were examined on removal from the 35° storage rooms for the degree of nesting and the percentage of berries rotted, and berries in the other half were examined after additional holding in air at 60° for 2 days to simulate shelf life in a store.

A taste test was run with strawberries. Only the darker red strawberries were used, to avoid any flavor difference because of differences in maturity. The berries were decapped, washed, and placed in the refrigerator until just before tasting began. Berries for tasting were placed on trays under Macbeth lights. Panelists (25 persons not experienced in strawberry tasting) tasted from two lots of treated berries and one lot of controls. Panelists were instructed to select at random one or two berries from each tray and taste them for flavor only. One panelist at a time entered the kitchen and made a judgment.

Test II.—Twelve pints of California strawberries (variety Z5-A) were used in a test in which a *Botrytis*-rotted berry was placed in the center of the top layer of berries in a basket as in test I. Half of the baskets were held at 60° F. and 90 percent RH for 3 days with 0.5 p.p.m. of ozone and half without ozone. The number of berries in a fungal nest was then determined.

Blueberries

Blueberries, free of decay and other defects, were placed 120 to a pint basket.

Test I.—Half of a 40-basket lot of berries was

⁴ See footnote 1.

⁵ See footnote 1.

held for 2 days at 35° F. and 90 percent RH with 0.5 p.p.m. of ozone and half without ozone. All berries were then held for 4 days in air at 70° and 90 percent RH. The berries were then examined for rot.

Test II.—Half of a 30-basket lot of berries was held for 6 days at 60° F. and 90 percent RH with 0.5 p.p.m. of ozone and half without ozone. The fruit was then examined for rot.

Cantaloups

Sixteen California cantaloups were placed in air at 45° F. and 90 percent RH and sixteen were placed in 0.5 p.p.m. ozone at the same temperature and humidity. After 7 days at 45°, the temperatures of the rooms were raised to 60° and after 5 days the cantaloups were examined.

Grapes

California Thompson seedless and California

Tokay grapes were inoculated by dipping the bunches in a beef extract-peptone broth suspension of *Botrytis cinerea* spores similar to the procedure used to inoculate strawberries. Ten bunches of each variety packed in wood flats were held in air and 10 bunches in ozone, both at 60° F. and 90 percent RH. The Thompson seedless grapes were removed from storage after 6 days and examined for appearance and rot. The Tokay grapes were examined after 7 days' storage.

Green Beans

Green beans were selected and divided at random into two samples. One sample was held in air and the other in 0.5 p.p.m. of ozone for 7 days at 45° F. and 90 percent RH followed by 5 days in air at 60° and 90 percent RH. Both samples were then examined for injury and decay.

RESULTS AND DISCUSSION

Peaches

Peaches of three different varieties inoculated with spores of *Rhizopus stolonifer* and *Monilinia fructicola* did not develop statistically significant differences in percentage of rhizopus rot or brown rot whether ozone was present or absent during storage for 1 week at 35° F. (table 1). Holding these peaches for 2 or 4 days at 70° without ozone did not reveal any effect of the ozone exposure on the subsequent shelf life of the peaches.

These studies confirm earlier results,⁶ which showed that ozone will inhibit the aerial growth of the mycelia of the rhizopus and brown rot fungi on peaches. When peaches were held for 1 week at 60° F., ozone reduced the nesting that occurs when *Rhizopus stolonifer* spreads from a *Rhizopus*-rotted peach to adjacent peaches by subsurface and aerial growth of mycelia (fig. 1). Although ozone significantly decreased the number of peaches in rhizopus and brown rot nests, considerable rotting occurred even with ozone treatment (table 2). When peaches are stored at 31° to 32°,⁷ nesting is not a problem, because the growth of fungi is inhibited by the low temperature. Higher temperatures, in which rot becomes a major problem, would be expected only when peaches are held for ripening or in display cases in stores. Ozone treatment would not be practical

TABLE 1.—Percentage of inoculated peaches that developed rhizopus and brown rot during storage in air or in ozone (0.5 p.p.m.) for 7 days at 35° F. and after a subsequent 2- or 4-day holding period without ozone at 70°¹

Holding period, temperature, and variety	Rhizopus rot		Brown rot	
	No ozone	Ozone	No ozone	Ozone
7 days at 35° F.	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Ranger.....	7	24	14	4
Keystone.....	15	17	12	14
Early Red Free.....	3	3	3	0
Mean ²	8	15	10	6
7 days at 35° F. +2 days at 70° F.				
Ranger.....	59	36	99	100
Keystone.....	25	22	75	72
Early Red Free.....	38	28	44	56
Mean ²	41	29	73	76
7 days at 35° F. +4 days at 70° F.				
Ranger.....	91	97	100	100
Keystone.....	36	40	99	100
Early Red Free.....	48	36	91	90
Mean ²	58	58	97	97

⁶ See footnotes 1 and 2.

⁷ WRIGHT, R. C., ROSE, D. H., and WHITEMAN, T. M. THE COMMERCIAL STORAGE OF FRUITS, VEGETABLES, AND FLORIST AND NURSERY STOCKS. U.S. Dept. Agr. Handb. 66, 77 pp. 1954.

¹ Each individual value for each variety is based on 100 peaches (5 baskets of 20 peaches each).

² Means for treatments with and without ozone are not significantly different at the 5-percent level.



FIGURE 1.—Influence of ozone on the development of nests of *Rhizopus stolonifer* (top row) and *Monilinia fructicola* (bottom row) in peaches: A, Peaches held in air for 7 days at 60° F. showing the abundant mycelia of *R. stolonifer* spreading throughout the peaches and showing the powdery spore masses of *M. fructicola*; B, peaches held under the same conditions as A but with 0.5 p.p.m. ozone added, showing reduced aerial mycelia of *R. stolonifer* and the ozone-induced spongy surface growth of *M. fructicola*. The slight reduction in the size of fungal nests due to ozone treatment is not readily seen.

BN-29472

TABLE 2.—Effect of ozone on the percentage of peaches in a basket that were infected by fungal spread from a RHIZOPUS- or MONILINIA-rotted peach during 7 days at 60° F.¹

Variety	Rhizopus rot		Brown rot (<i>Monilinia</i>)	
	No ozone	Ozone	No ozone	Ozone
	Percent	Percent	Percent	Percent
Early Red Free.....	84	67	66	48
Elberta.....	69	61	37	20
Rio Oso Gem.....	79	52	50	40
Mean ²	77 a	60 b	51 b	36 c

¹ Each individual value for each variety is based on 100 peaches (5 baskets of 20 peaches each).

² Means not followed by the same letter are significantly different at the 5-percent level.

in open display cases, but might have limited value in ripening rooms.

Confirming previous results,⁸ ozone at 0.5 p.p.m. did not injure the peaches.

Strawberries

The development of fungal nests in strawberries to which a *Botrytis*- or *Rhizopus*-rotted berry had been added as a nesting center was inhibited at 35° F. regardless of the presence or absence of ozone (fig. 2). Leakage from the *Rhizopus*-rotted berry caused an enzymatic breakdown of adjacent berries which ozone could not prevent. Ozone did not significantly reduce the percentage of botrytis or rhizopus rot during 7 days of storage at 35° nor improve the shelf life of the strawberries (table 3).

⁸ See footnote 1.

Storage at 31° to 32°, as recommended,⁹ would almost completely eliminate fungal growth.

These results confirm previous data,¹⁰ which show no statistically significant effect of ozone on strawberries.

On one occasion, berries tasted during an examination immediately after removal from ozone storage had a fresh taste, while berries removed from the control room had a bad storage flavor. In a test run on a subsequent lot of strawberries, a taste panel was unable to detect a significant difference in flavor a few hours after the fruit was removed from the storage rooms. Any flavor difference existing on removal apparently disappeared rapidly.

An additional experiment was conducted to determine the influence of ozone on fungal

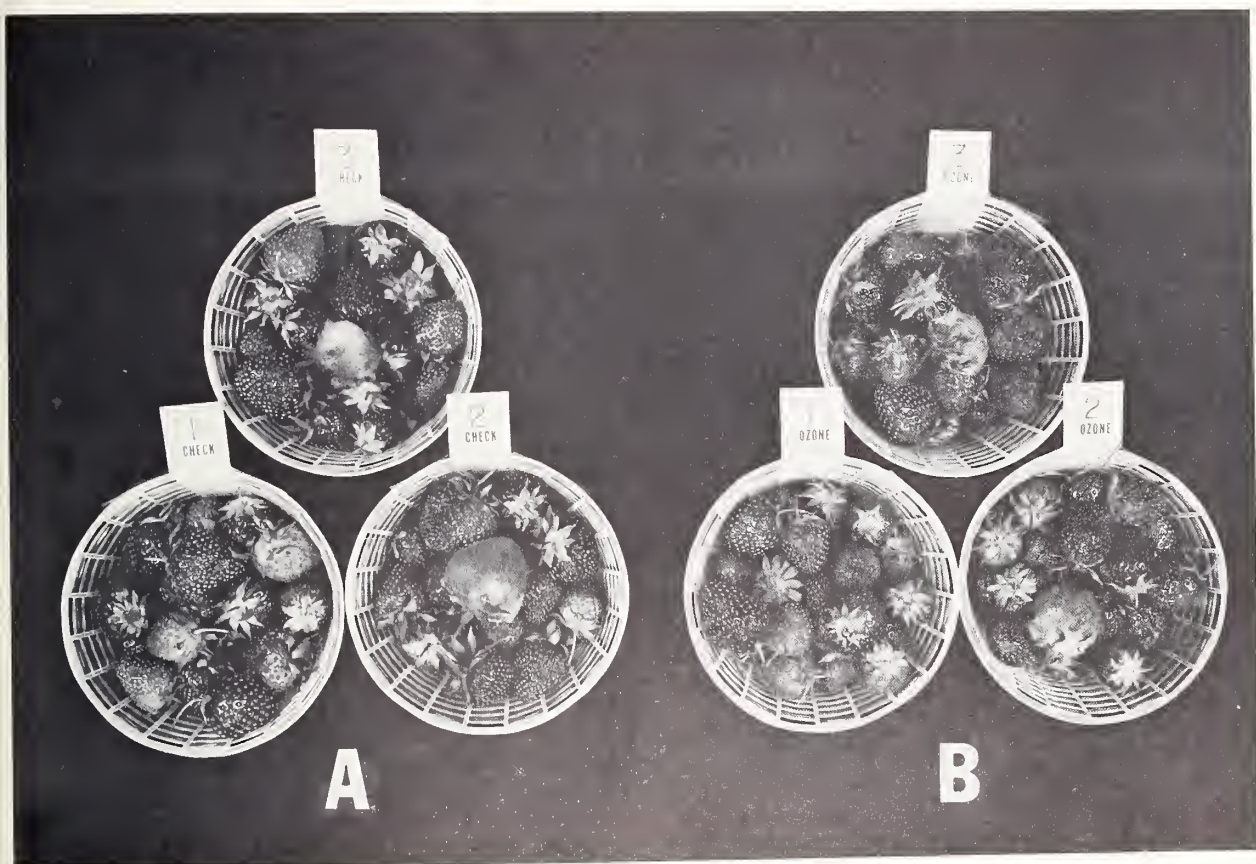
nesting in strawberries held at 60° F. Under these conditions, ozone reduced the number of berries in nests developing in baskets of strawberries to which a *Botrytis*-rotted berry had been added (fig. 3). The nests contained an average of 52 percent of the berries in a basket held without ozone and 23 percent when held in ozone. In ozone the nest was confined largely to berries in contact with the rotted berry serving as a nesting center. Additional experiments were not made at this temperature because berries are not held commercially under these conditions except in display cases in stores where ozone could not be used. As mentioned earlier, the problem of nesting can be effectively controlled by temperature.

Ozone at the 0.5 p.p.m. concentration used in these studies injured the caps of strawberries and caused the caps to shrivel and dry, confirming previous data.¹¹

⁹ See footnote 7.

¹⁰ See footnotes 1 and 3.

¹¹ See footnote 1.



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FIGURE 2.—Influence of ozone on the development of nests of *Botrytis cinerea* (treatment 2) and *Rhizopus stolonifer* (treatment 3) in strawberries: A, Strawberries held in air for 7 days at 35° F., showing the lack of fungal spread from the original nesting centers (mold-covered berries); B, strawberries held under the same conditions as A but with 0.5 p.p.m. ozone added appear essentially identical to A.

TABLE 3.—Percentage of strawberries that developed rhizopus and botrytis rot during storage for 7 days in air or in ozone (0.5 p.p.m.) at 35° F. and after a subsequent 2-day holding period at 70° without ozone ¹

Holding period, temperature, and variety	Treatment 1 ²		Treatment 2 ²		Treatment 3 ²	
	No ozone	Ozone	No ozone	Ozone	No ozone	Ozone
7 days at 35° F.	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>	<i>Percent</i>
Sel. 2929.....	36	57	73	73	68	64
Do.....	45	35	49	42	48	51
Dixieland.....	0	20	17	24	22	28
Sel. 3201.....	29	21	31	25	33	39
Do.....	21	19	41	24	43	29
Mean ³	26 a	30 a	42 b	38 b	43 b	43 b
7 days at 35° F. + 2 days at 60° F.						
Dixieland.....	68	52	60	68	62	77
Sel. 2929.....	79	79	91	79	77	79
Sel. 3198.....	79	80	82	89	84	88
Sel. 3201.....	80	69	98	76	84	71
Do.....	70	63	86	94	83	71
Mean ³	75 a	68 a	83 b	81 b	78 ab	77ab

¹ Each mean is an average of the percent rot in 10 pint baskets.

² In treatment 1, a *Botrytis*-rotted berry was added to each basket as a nesting center. In treatment 2, a *Rhizopus*-rotted berry was added. No rotted berry was added in treatment 3.

³ Means for each holding period not followed by the same letter are significantly different at the 5-percent level.

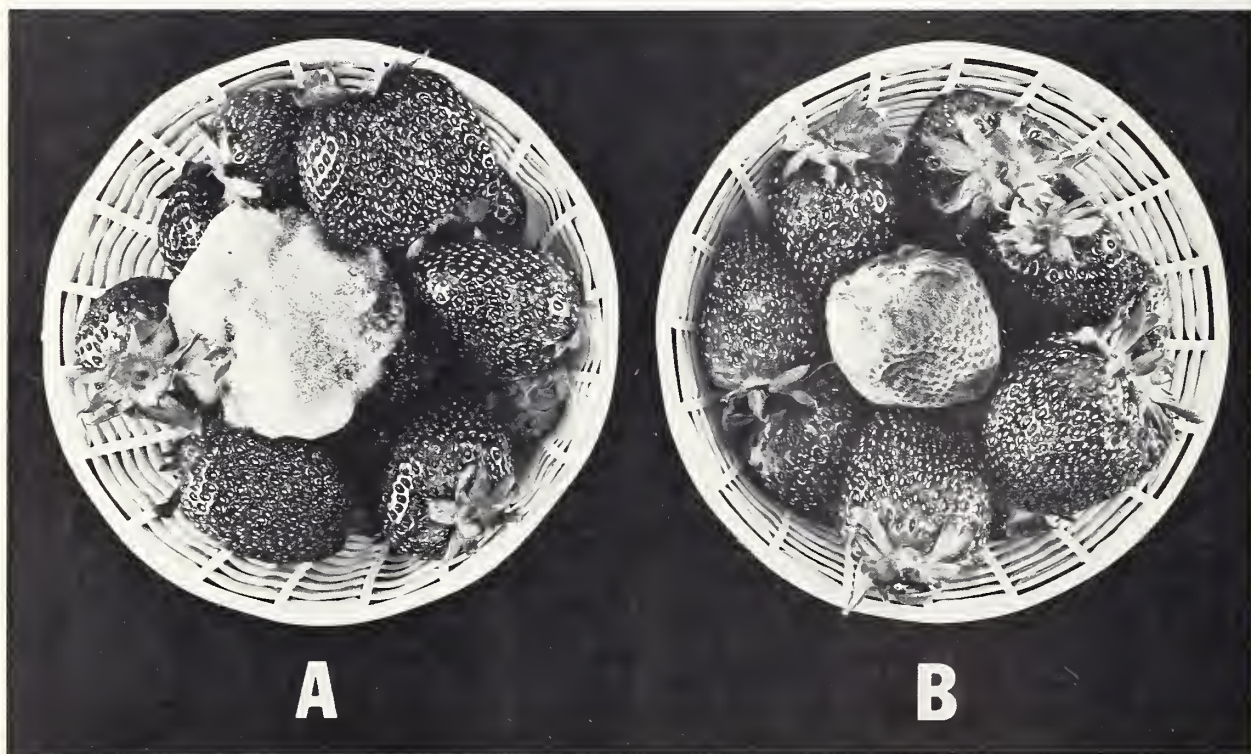


FIGURE 3.—Influence of ozone on the development of nests of *Botrytis cinerea* in strawberries: A, Strawberries held in air for 3 days at 60° F. and 90 percent relative humidity, showing early stage of fungal nest development in which the mycelia of *B. cinerea* are spreading to adjacent berries; B, strawberries held under the same conditions as A but with 0.5 p.p.m. ozone added, showing the dead shriveled aerial mycelia of the fungus restricting spread of the fungus to points of contact between healthy and infected tissue.

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Blueberries

The commercial shipping and market retailing of blueberries was simulated by holding the berries for 2 days at 35° F. followed by 4 days at 70°. Application of ozone during the 2 days at low temperature did not influence the subsequent shelf life of the fruit. Thus, at the end of the 4 days at 70°, the mean percentage of blueberries rotted was 20 percent in berries held in ozone and 18 percent in fruit not held in ozone (table 4).

TABLE 4.—Percentage of blueberries that developed rot during storage in air or in ozone (0.5 p.p.m.) for 2 days at 35° F. and after a subsequent 4-day holding period without ozone at 70°

Replicate ¹	No ozone	Ozone
	Percent	Percent
1.....	15	17
2.....	22	23
Mean ²	18	20

¹ Each treatment in replicate 1 consisted of 12 baskets, each containing 120 berries. Replicate 2 consisted of 8 baskets per treatment.

² Means are not significantly different at the 5-percent level.

Although low temperature is recommended for storage of blueberries, tests were also run at 60° F. After 6 days, 21.8 percent of the blueberries held in ozone were rotted and 25.6 percent of those without ozone. The difference was not statistically significant at the 5-percent level. The berries stored in ozone, however, had less surface mold growth than the check fruit.

Cantaloups

Cantaloups held for 7 days at 45° F. followed by 5 days at 60° developed the same amount of rot (68.7 percent) regardless of the presence or absence of ozone during storage. Ozone inhibited the surface growth of *Penicillium* spp. and *Alternaria tenuis* auct. The internal appearance of the fruit was not influenced by ozone, but fruit held in ozone had a slightly better external appearance because of less surface mold.

Grapes

After 6 days at 60° F., gray-mold rot (caused by *Botrytis cinerea*) of Thompson seedless

grapes held in air without ozone was 82.6 percent, while rot in fruit held in ozone was 84.7 percent or essentially the same as in air. Although superficial mold growth was inhibited by ozone (fig. 4), the browning of the skin and easy skin-slipping characteristic of gray-mold rot in grapes was still evident in grapes stored in ozone. Most of the ozone apparently did not reach the underlying layers of grapes since the grapes on the undersides of the bunches had surface mold identical to that found in control fruit.

Similar results were obtained with Tokay grapes examined after 7 days at 60° F. The surface appearance of the grapes stored in ozone was more attractive because of less surface mold growth. However, when the bunches were turned over, there was just as much rot with surface mycelial growth in the ozone-treated as in the check grapes (fig. 5). The check fruit had 83.7 percent rot and the treated fruit had 81.2 percent rot.

These results with grapes support unpublished data of Uota and Harvey,¹² which showed sulfur dioxide to be superior to ozone in reducing gray-mold rot of grapes.

Green Beans

When green beans were stored in air for 7 days at 45° F., then held for 5 days at 60°, 33.2 percent developed alternaria rot; 29.5 percent of the beans treated similarly but in an atmosphere containing ozone developed rot. The difference in the percentage of rot is not statistically significant. Alternaria rot (caused by *Alternaria tenuis*) did not develop until the beans had been weakened by the long storage.

Ozone injured 20 percent of the beans. The injury appeared as blotchy brown areas of killed tissue (fig. 6) and was apparent by the end of the 7 days at 45°. Only the outer layers of beans showed damage, illustrating the poor penetrative powers of ozone because of its rapid breakdown when in contact with organic matter.¹³

¹² Unpublished data of a test conducted in 1963 by M. Uota and J. Harvey of the Horticultural Crops Research Branch, Market Quality Research Division, Fresno, Calif.

¹³ EWELL, A. W. OZONE AND LIGHT: II. OZONE. In Refrigerating Data Book, Refrigeration Application Volume, Ed. 2, Sec. II. Cold Storage Practice, ch. 18, pp. 196-199. Amer. Soc. Refrig. Engin., Menasha, Wis. 1946.

MALLISON, E. D., and SPALDING, D. H. USE OF OZONE IN TOMATO RIPENING ROOMS. U.S. Dept. Agr. ARS 52-17, 10 pp. 1966.

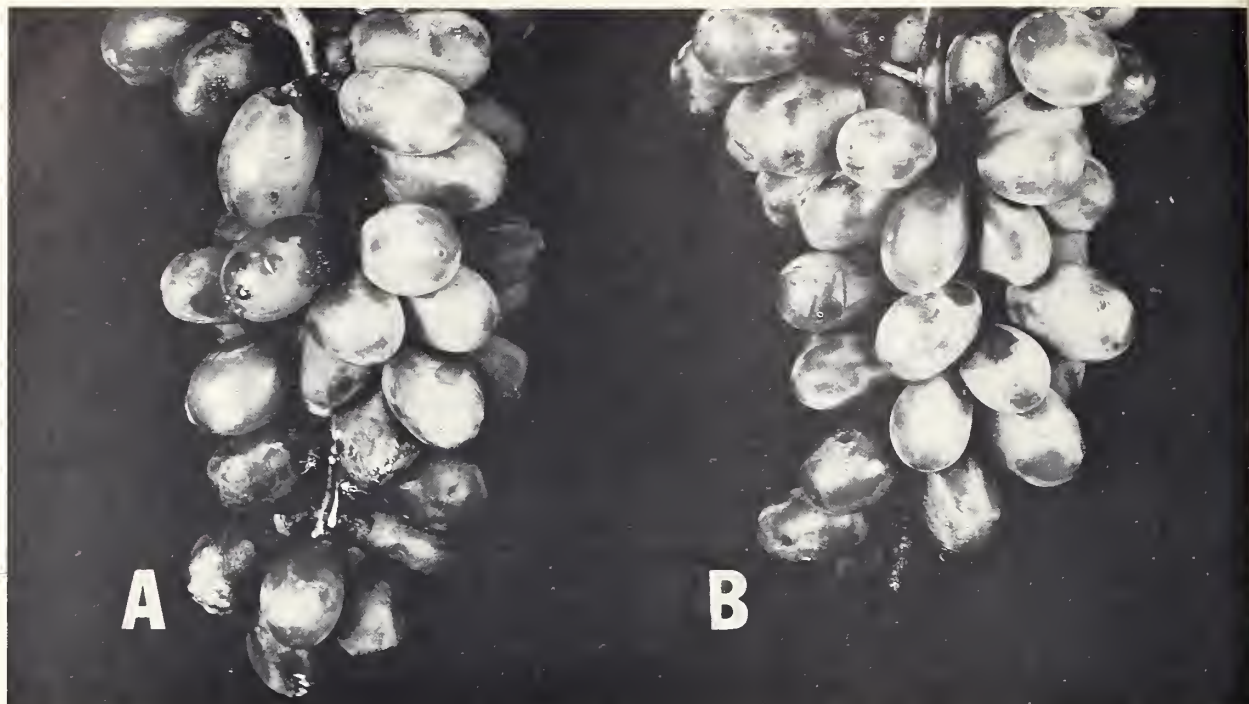


FIGURE 4.—Influence of ozone on the development of gray-mold rot of Thompson seedless grapes: A, Grapes held in air for 6 days at 60° F., showing growth and sporulation of *Botrytis cinerea* especially abundant at the stem ends; B, grapes held under the same conditions as A but with 0.5 p.p.m. ozone added, have the same amount of rot but have a better appearance because of suppression of mold growth by ozone.

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FIGURE 5.—Influence of ozone on the development of gray-mold rot of Tokay grapes in typical bunches from the top layer of the flat (upper) and from the bottom layer of the flat with the latter bunches turned upside down (lower): A, Grapes held in air for 7 days at 60° F. showing *Botrytis cinerea* mold covering the upper surface of grapes in the top layer and covering the lower surface of grapes in the bottom layer; B, grapes held under the same conditions as A but with 0.5 p.p.m. ozone added, showing the ozone suppression of surface mold on the upper surface of grapes from the top layer contrasted with the mold-covered lower surfaces of grapes from the bottom layer and demonstrating the inability of ozone to penetrate the layers of a pack.

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FIGURE 6.—Ozone injury to green beans: *A*, beans held for 7 days at 45° F.; *B*, beans held under the same conditions as *A* but with 0.5 p.p.m. ozone added show blotchy patches of injured tissues, which are light brown in color.

