

Figure 2. R_f in phenol plotted against reciprocal of number of carbon atoms in amino acid chain

Wieland and Wirth (14). Judging from the difference in the rate of degradation of D,L-threonine and L-threonine, there is a structural factor controlling the alkaline degradation of threonine. These re-

actions appear worthy of further attention.

In addition to loss in threonine and serine during liming, it seemed that one of the unidentified amino acids which has an R_f near that of β -alanine disappeared. A test to determine whether the alanine undergoes β -elimination was negative. Ornithine has been investigated and found to form two new ninhydrin reacting compounds which have not been identified, although one is probably a pyrrolidone complex. Similarly, γ -aminobutyric acid diminishes in quantity on heating in mild alkaline solutions.

It is apparent that alkaline hydrolysis of protein or alkaline treatment of amino acids is likely to cause marked changes among the amino acids and such treatment should be checked for its effect before conclusions are drawn from subsequent analyses. Removal of ammonia by rapid evaporation apparently has very little effect on threonine, as shown in Table IV.

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GREEN LEMON MOLD Gaseous Emanation Products

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Common green lemon mold (*Penicillium digitatum*) produces ethylene, which powerfully stimulates lemon respiration. An irradiated mutant mold failed to stimulate lemon respiration. The chemical differences of the emanations from the two molds were investigated. Vapors from moldy lemons were collected by activated carbon adsorption, desorbed in vacuo, and analyzed by mass and infrared spectra. The mutant mold produced less total volatile material than the nonmutant mold, and there were significant qualitative chemical differences between the two emanations. The stimulating effect of *Penicillium digitatum* on respiration of healthy lemons can be reduced by purifying the air contaminated by the mold gases with brominated activated carbon. An alternative method consists of simultaneous diffusion of small concentrations of bromine into the storage space and recirculation of the storage air through unbrominated carbon.

THE GASEOUS EMANATIONS from common green lemon mold (*Penicillium digitatum*) have been shown (3) to contain a powerful stimulant for lemon

respiration, probably ethylene. Figure 1, taken from Biale and Shepherd (3), illustrates the course of this stimulation. The evidence that the stimulating gas is,

in fact, ethylene includes the following: The stimulation of lemon respiration by pure ethylene is similar to that produced by gaseous emanations from the mold;

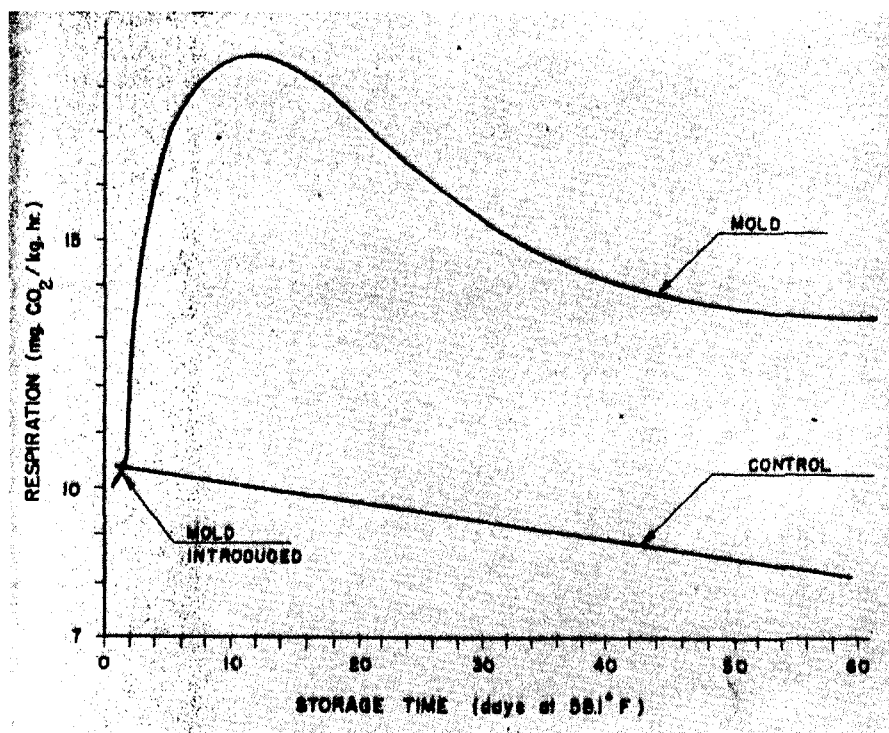


Figure 1. Respiration of lemons as affected by vapors of one moldy lemon *P. digitatum*

the mold emanation gives a positive epinasty test; and the mold emanation, after being scrubbed with 95% sulfuric acid, is absorbed by mercuric perchlorate in perchloric acid solution to form an addition product. Finally, this addition product yields a gas (ethylene) which, when brominated, produces ethylene dibromide, which can be chemically identified with an authentic sample (15).

A mutant mold prepared by ultraviolet irradiation failed to stimulate lemon respiration (2). Therefore, the mutant mold presumably produces no ethylene.

The gaseous emanations from the two molds were collected for more complete analysis by adsorption on activated carbon. The source of the carbon was coconut shells; its properties corresponded to the specifications for activated carbon for gas adsorption given in Table I.

To collect the vapors, pure air saturated with water was passed over 18 lemons inoculated with the ethylene-producing strain, and then through three sections of activated carbon in series. The first section contained 10 grams of carbon, the second and third contained larger quantities. The air was passed over the fruit and thence through the carbon at a rate of 350 ml. per minute for 77 hours. At the start of the run, the fruits were three-fourths covered with mold and at the end they were completely covered. The moldy fruit and the carbon were maintained at 25° C. throughout the run. Vapors from the mutant non-ethylene-producing strain were collected by a parallel procedure. The adsorbed gases were removed from the carbon by high vacuum desorption. In each case, only the initial section of carbon (10 grams) contained adsorbed matter; the second and third sections were therefore discarded. Thus, the

adsorption was quantitatively efficient and the adsorptive capacity of each 10-gram quantity of carbon was not exceeded.

Vacuum Desorption of Carbon

Vacuum desorption was carried out in the apparatus shown in Figure 2. The carbon from the ethylene-producing mold (13.5 grams) was placed in the desorption flask, the traps were filled with refrigerants, and the system was evacuated with the mechanical forepump. Stopcocks C and E were closed. After 0.5 hour the diffusion pump was started and pumping continued for 3 hours at a pressure of about 1 micron. The tem-

Table I. Specifications for Activated Carbon for Air Purification

| Property | Specification |
|---------------------------------------|------------------------|
| Activity for CCl_4^a | At least 50% |
| Retentivity for CCl_4^b | At least 30% |
| Apparent density | At least 0.42 gram/ml. |
| Hardness (ball abrasion) ^c | At least 80% |
| Mesh distribution | 6-14 range (Tyler) |

^a Maximum saturation of carbon, at 20° C. and 760 mm., in air stream equilibrated with CCl_4 at 0° C.

^b Maximum weight of adsorbed CCl_4 retained by carbon on exposure to pure air at 20° C. and 760 mm.

^c Per cent of 6-8-mesh carbon which remains on 14-mesh screen after vibrating with 30 steel balls of 0.25 to 0.37-inch diameter per 50 grams of carbon, for 30 minutes

perature of the heating mantle was 250° to 300° C. At the end of the pumping period, the system was slowly returned to atmospheric pressure by bleeding air in through stopcock C. Stopcocks A and B were then closed and the traps were removed and examined. (Caution. In following this procedure, care must be exercised to avoid condensing atmospheric oxygen in the liquid nitrogen trap. If necessary, the trap should be warmed to dry ice temperature before bleeding in air.)

The ice salt trap contained only water. This was discarded. The dry ice trap

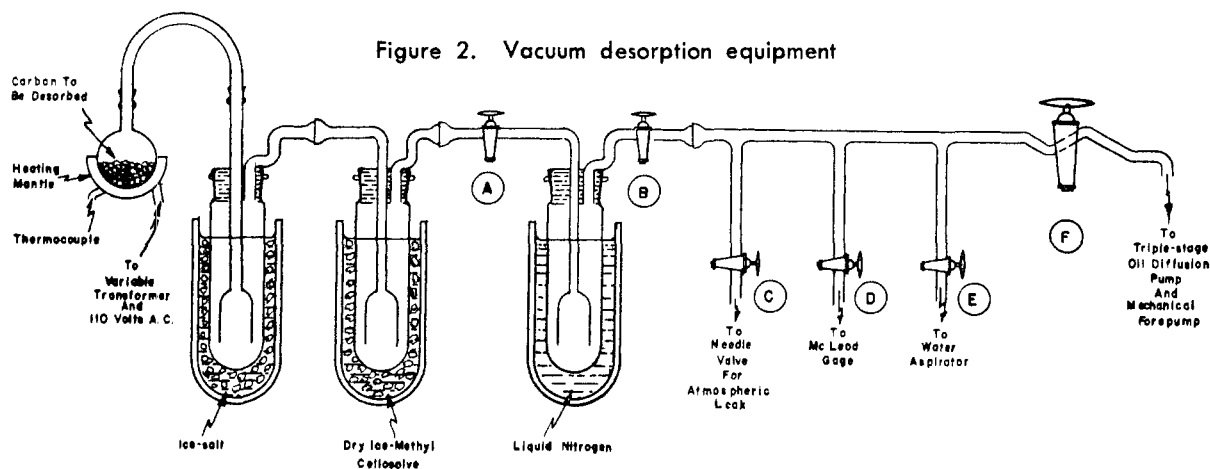


Figure 2. Vacuum desorption equipment

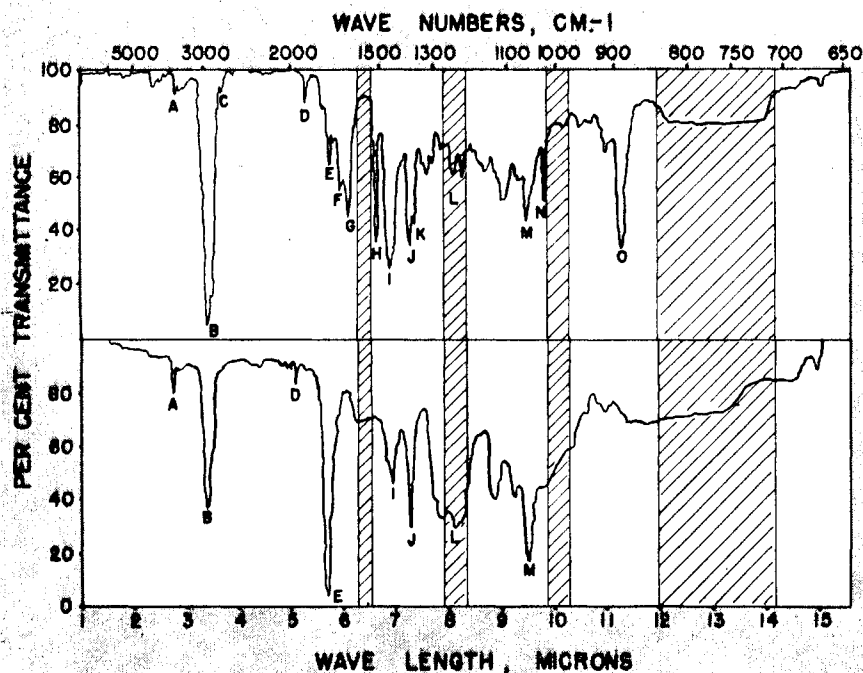


Figure 3. Infrared spectra

Above. Vapors from *P. digitatum* (lemon mold) collected on carbon
Below. Vapors from mutant of *P. digitatum* collected on carbon

contained water with a small surface film of oily matter. The liquid nitrogen trap contained a few drops of organic liquid. The weight of the desorbed carbon was 9.7 grams. Therefore the degree of saturation of the carbon was $(13.5 - 9.7)/9.7$, or 39.2%, based on original carbon. Most of this was water.

Vacuum desorption of the carbon from the mutated non-ethylene-producing mold (13.2 grams) was carried out by a similar procedure. The quantities of organic material in the dry ice and liquid nitrogen traps were substantially less than those obtained from the preceding desorption. The weight of the desorbed carbon was 10.1 grams. Therefore, the degree of saturation of the carbon

was $(13.2 - 10.1)/10.1$ or 32.6%, based on original carbon. Again, most of this was water.

Infrared Spectral Analysis (13)

Each of the two samples collected in the dry ice traps was extracted with about 2 ml. of carbon tetrachloride. The carbon tetrachloride solution was then dried with such a small quantity of anhydrous calcium chloride that an aqueous layer of calcium chloride monohydrate was formed upon dehydration of the organic layer. In this way the dried carbon tetrachloride solution was not in contact with solid granular calcium chloride, which might otherwise adsorb some of the material to be analyzed.

The solution of the gases desorbed from the ethylene-producing mold was then subjected to infrared analysis. The instrument used was a double-beam infrared spectrophotometer (Model 21, Perkin-Elmer Corp., Norwalk, Conn.); the thickness of the film in the absorbing cell was 0.5 mm.; the compensating beam was passed through pure carbon tetrachloride in a variable-space cell.

The solution of gas from the mutated non-ethylene-producing mold was similarly analyzed. Because this solution was more dilute, however, a cell thickness of 2 mm. was used.

Figure 3 shows the spectra of gases from the two molds, collected in dry ice traps from carbon desorption. The cross-hatched sections mark off wave lengths in which the carbon tetrachloride solvent absorbs, and which are therefore analytically unreliable. The labeled absorption peaks correspond to functional groups and molecular patterns as follows (7):

- A. —O—H (hydroxyl)
- B. —C—H stretching (organic compounds generally)
- C. —C=O (aldehyde)
- D, G, H. Conjugated double bond structure (aromatic)
- E. —C=O (ester or aldehyde)
- F. C=C (unsaturation, not conjugated)
- I. —C—H bending (organic compounds generally)
- J. —CH₃ (terminal methyl groups)
- K. —CH₃ (side-chain methyl groups)
- L. =C—O (esters)
- M. —C—O (alcohol, ether, or aldehyde)
- N, O. C=C (unsaturation at terminal portion of molecule)

It is at once apparent that the spectrum of vapors from the mutant mold shows a smaller total quantity of evolved volatiles and significant qualitative changes. It is important to keep in mind that these observed differences may be related either to qualitative or quantitative differences in the vapors from the

Figure 4. Mass spectrum of mold vapors

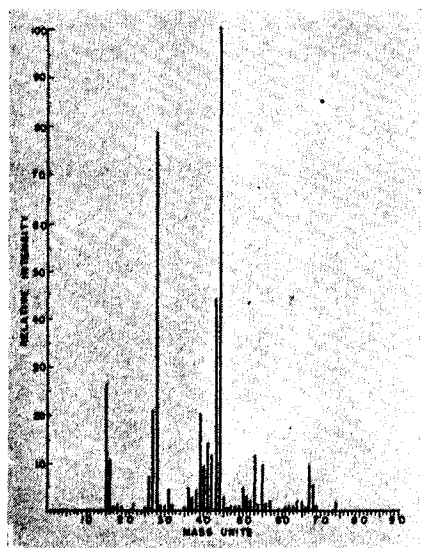


Figure 5. Mass spectrum of mold vapors

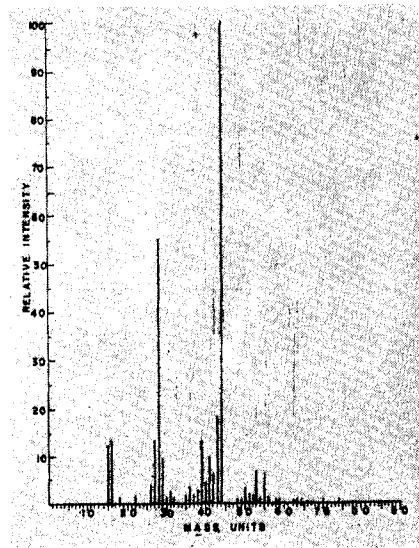
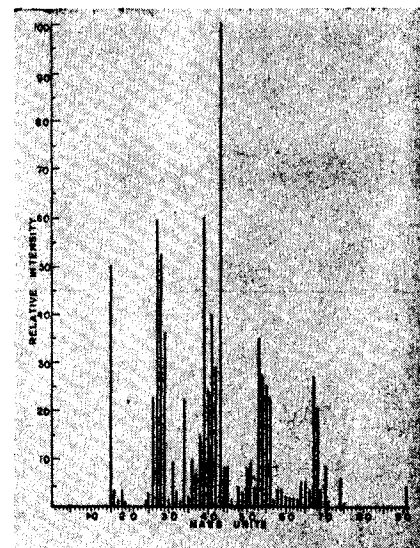


Figure 6. Mass spectrum of mold vapors



two molds (because of their genetic dissimilarity) or to differences in the gases given off by the lemons themselves (because of their lesser stimulation by the mutated mold). A more rigorous interpretation might be facilitated by a second series of experiments, which examined the vapors from healthy lemons in pure air and from healthy lemons stimulated by air containing a small amount of pure ethylene. It might then be valid to assume that:

Spectrum of gases from ethylene-producing mold on lemons — spectrum of gases from healthy lemons equally stimulated by pure ethylene = spectrum of gases from ethylene-producing mold (1)

Spectrum of gases from mutant mold on lemons — spectrum of gases from healthy lemons in pure air = spectrum of gases from mutant mold (2)

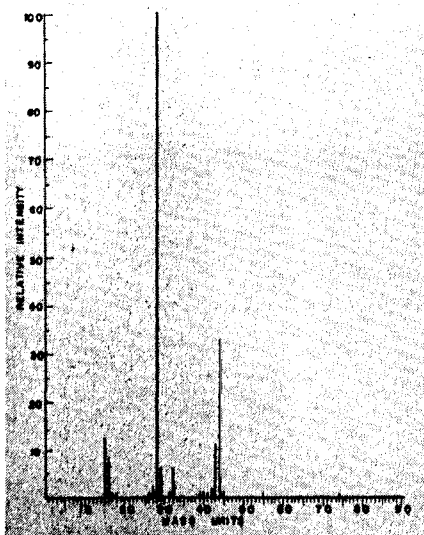
However, if the simplifying assumption is made that the lemon gases are not qualitatively changed when their respiration rate is increased, then the qualitative differences shown by Figure 3 must be attributed to the genetic dissimilarities of the two molds.

Most striking is the elimination of peaks characteristic of unsaturation from the spectrum from the mutated mold; these include *F*, *N*, and *O*, corresponding to internal and terminal double bonds. Thus, the mutation eliminates not only ethylene but also its homologs and derivatives from the gaseous mold emanations. Other effects include elimination of peaks *K* (side-chain methyl groups), *G* and *H* (conjugated unsaturation, probably aromatic), and almost all of *C* (aldehyde). On the other hand, the spectrum from the mutated mold shows a much more prominent peak *E* (carbonyl, probably ester).

Mass Spectral Analysis

The materials isolated in the liquid nitrogen traps (Figure 2) were used for mass spectral analysis.

Figure 7. Mass spectrum of mold vapors



Progressively less volatile samples were subjected to analysis, according to the fractionation procedure of Table II.

The spectra shown in Figures 4 to 10 represent the difference between the observed spectrum and the background spectrum of the instrument used. The following interpretations are made (7, 8, 17):

As the fractionations produced progressively less volatile samples, the prominence

of peaks 28 (nitrogen) and 44 (carbon dioxide) diminished (Figures 4 to 6 and 7 to 10).

For both molds, the prominent peaks (discounting normal atmospheric gases) include masses:

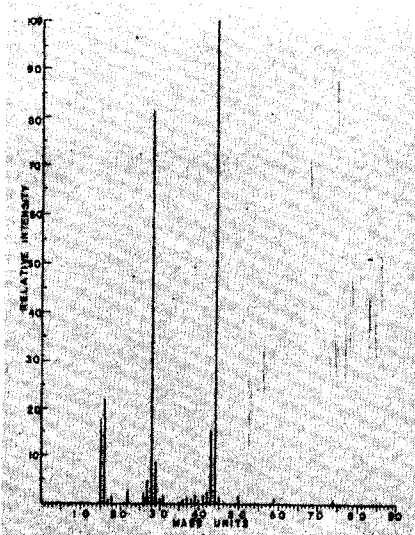
- 26. C_2H_2 (ethyl groups)
- 27. C_2H_3 (ethyl groups)
- 29. CHO (aldehydes, formic esters, alcohols)
- 31. CH_3O (primary alcohols, formic esters)
- 43. CH_3CO (acetate esters)
- 45. CH_3-CHOH (secondary alcohols)

Assignments of molecular fragments to heavier peaks become less unique and are therefore avoided.

Some peaks prominent in the spectra of the ethylene-producing mold are conspicuously weak in the spectra from the mutant mold—e.g., 37–41, 46–56, 64–65. Because of the complexity of the mixtures and the absence of more detailed knowledge of the possible compounds present, specific formulas corresponding to these masses cannot be assigned.

Typical compounds which may be present in the nitrogen-trapped gases from both molds include acetaldehyde, *n*- and isopropyl alcohols, methyl acetate, and

Figure 8. Mass spectrum of mold vapors



ethyl formate. Their heavier homologs would not have been likely to pass beyond the dry ice trap (Figure 2) during the carbon desorption.

For more complete analysis of such vapors it would be necessary to isolate larger quantities of material by the carbon sorption-desorption method, fractionate the desorbate by chromatographic or other means, and examine the pure compounds or simpler mixtures thus obtained.

Control of Mold-Stimulated Lemon Respiration

The accelerated lemon respiration caused by the ethylene emanating from common green lemon mold can be effectively controlled by purifying the storage air with bromine and activated carbon.

It has been shown (4–6) that unbrominated activated carbon does not effectively control ethylene concentration in a fruit storage atmosphere. This is not surprising when one considers the characteristics of physical adsorption by activated carbon of a gaseous atmospheric contaminant with a molecular weight equal to that of air (9). An early remedy involved the use of carbon impregnated with 15% or more of its weight of bromine (10). The circulation of fruit storage air through beds of such bromine-impregnated carbon effectively removes ethylene, but at the expense of serious practical disadvantages: Brominated carbon is very corrosive and therefore requires expensive containers to hold it in uniformly thick beds and brominated carbon is hygroscopic to an extent which depends on its degree of impregnation. In humid atmospheres, the carbon surface may even become wet. It has recently been shown (14) that these properties are a consequence of the rapid hydrolysis of free bromine on the carbon surface. To take advantage of the carbon-catalyzed reaction be-

Figure 9. Mass spectrum of mold vapors

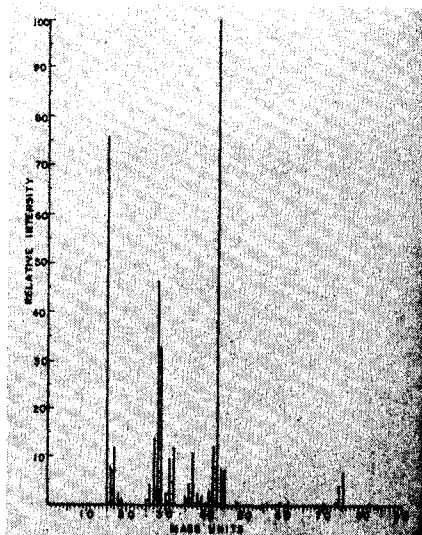


Table II. Fractionation Procedure

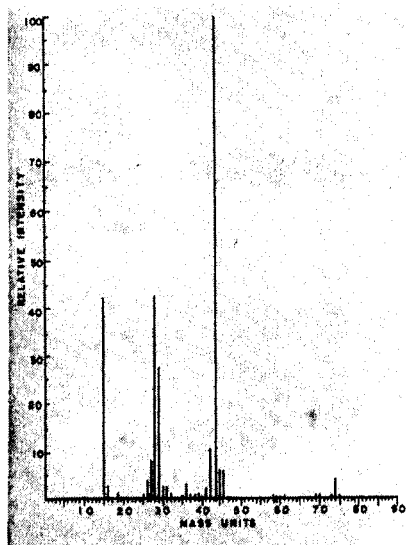
| Sampling Method | Total Pressure of Sampled Gas, Cm. | | Mass Spectrum | | Remarks |
|---|------------------------------------|-------------|---------------|-------------|---|
| | Ethylene mold | Mutant mold | Ethylene mold | Mutant mold | |
| Contents of trap connected directly to mass spectrometer sampling tube | 4.0 | 4.0 | Fig. 5 | Fig. 8 | Lightest gases, including air |
| Material remaining in trap frozen with liquid N ₂ ; gas pumped from trap; trap then connected to spectrometer sampling tube and warmed to room temperature | 4.0 | 4.0 | Fig. 6 | Fig. 9 | Gases not volatile at liquid N ₂ temperature |
| Material remaining in trap frozen with dry ice; gas pumped from trap and residue warmed and sampled as above | 4.2 | 7.4 | Fig. 7 | Fig. 10 | Gases not volatile at dry ice temperature, include most organic volatiles |
| Material remaining in trap frozen in ice water; gas pumped from trap and residue warmed and sampled as above | | 1.8 | | Fig. 11 | Gases not volatile at 0° C. |

tween ethylene and bromine and at the same time avoid the practical difficulties noted above, a system was set up in which the bromine was slowly diffused into the test space, from which the air was circulated through unbrominated activated carbon. This system is more fully described elsewhere (12). The experimental procedure duplicated that described by Biale and Shepherd (3), with the modifications noted:

Mold-Stimulated Lemons. Purified humidified air was passed at 500 ml. per minute over three moldy lemons and thence to 2.65 kg. of healthy lemons at 55° F.

Mold Vapors Plus Bromine Plus Car-

Figure 10. Mass spectrum of mold vapors

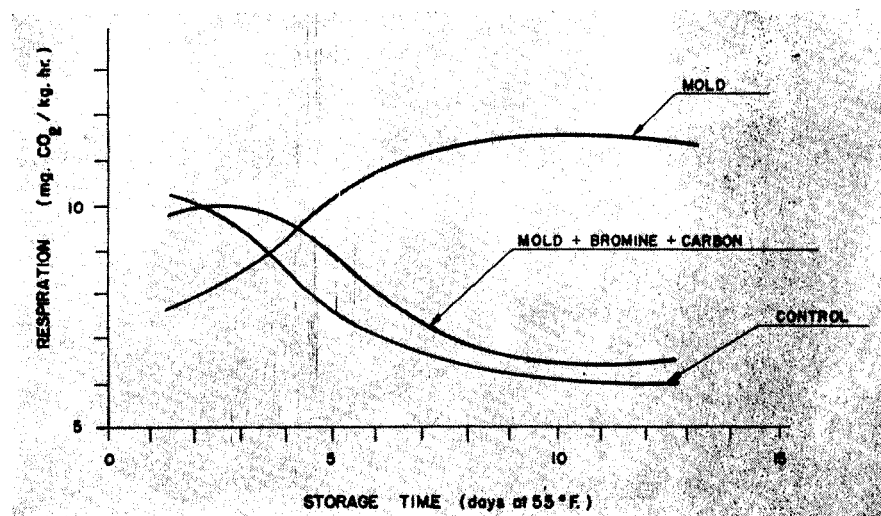


bon. Purified humidified air was passed at 500 ml. per minute over three moldy lemons; bromine was then allowed to diffuse into the contaminated air stream, so that the concentration of bromine in the air did not exceed 50 p.p.m.; the air was then passed through a bed of activated gas adsorption carbon 1 inch thick (Table I) and thence to 2.60 kg. of healthy lemons at 55° F.

Control. Purified humidified air only was passed directly to 2.72 kg. of healthy lemons at 55° F.

Figure 11 shows the respiration rate of the three lemon samples. The bromine and carbon treatment affords effective control of the stimulation which the mold would otherwise produce. The entire range of effective bromine concentrations was not explored.

Figure 11. Control of mold-stimulated lemon respiration



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