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Acknowledgment

The authors wish to express their appreciation to E. R. Cousins and Weaver Landmann for assistance in the preparation of the hydrogenated wax, to Alva Cucullu and V. O. Cirino for many of the analytical determinations, to W. H. Hodge, Plant Introduction Section, for assistance in obtaining the seed for this work and for supplying the names of parties interested in evaluating the products, and to Bernard Benson, Boyce Thompson Arboretum, Superior, Ariz., for obtaining the seed.

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TOXIC GASES IN SILAGE

Production of Toxic Gas (Nitrogen Oxides) in Silage Making

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Because several deaths and serious illnesses have resulted from exposure to nitrogen dioxide produced in silage making, the attention of agricultural experimentation workers has been sharply focused on this problem. Five silos were tested in the summer and fall of 1956 for nitrogen dioxide production; all gave off the gas. In one instance the brown fumes were so extensive that good colored photographs of the gas were obtained. Many lots of experimental silage have been prepared to determine the factors involved in the production of nitrogen oxides in silage.

PRODUCTION OF THE TOXIC GAS, NITROGEN DIOXIDE, in the early stages of silage making has become recognized in recent years as a serious and insidious danger. Although previous reports of chickens found dead at the bottom of the silo chute had come to the College of Agriculture, no definite leads were obtained until September 1949, when a sample of silage in a closed bottle was received from a farmer who reported that he had been almost overcome trying to climb the ladder in the chute of his silo. When opened, the

bottled silage gave off a strong bleach-like odor, had a peculiar orange color, and gave strong color tests for nitrites and nitrates.

A day or two later, one of the University Farm silos gave off a clearly visible brownish gas which accumulated in the unventilated chute of the silo and analyzed several hundred parts per million of nitrogen dioxide. Visible gas was not observed from any other silo that year, but evidence of its formation was indicated by the presence of orange to brown colored pieces of forage which gave positive nitrite tests. Following publication of results of these tests (7, 15, 16), letters from farmers and experiment station workers in Minnesota, South Dakota, Pennsylvania, and Wisconsin, were received telling of the occurrence of the gas in these regions and of its causing the death of some farm animals.

Medical Reports

Real impetus toward a recognition of the seriousness of nitrogen oxides in silage making came in 1955, when farmers in several parts of the country were brought to hospitals suffering from a mysterious pulmonary illness that could not be related to any known cause. In all cases the men had become violently ill, with coughing and shortness of breath, after going up into a silo soon after it was filled or partly filled with

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forage. The first report on two such cases was made by Delaney, Schmidt, and Stroebel (5) in April 1956. A careful and systematic study of the patients, one of whom died, and considerations of the report on the production of nitrogen dioxide in silage (7), led to the conclusion that the illnesses were similar to those caused by nitrogen dioxide poisoning.

Four subsequent cases, two of which ended fatally, were reported by Lowry and Schuman (11) and two others, one fatal, by Grayson (9). In several cases irritating fumes and even a yellowish brown gas in the silo chute or above the silage was noted at the time the men entered the silos. All these cases have been reported in great detail with respect to both clinical and laboratory findings. Illness caused by exposure to nitrogen dioxide gas from silos is now designated by medical men as "silo-filler's disease" or "chemical pneumonia."

Literature Review on Production of Nitrogen Oxides

While the production of nitrogen dioxide in silage fermentation is currently regarded as new and strange, it is really an old and well-recognized type of fermentation—i.e., large scale denitrification under specialized conditions. Production of nitrogen dioxide in fermenting beet molasses was reported by Dubrunfaut (6) in 1868. Formation of the gas in stored molasses was reported recently by Verhoeven (19) and has been observed in the United States (7).

Schloesing (17) reported production of nitrogen dioxide from the juice of tobacco plants and from urine, when these materials underwent spontaneous fermentation, and Gayon and Dupetit (8) obtained it by addition of nitrates to molasses, plant juice, and other natural materials. The latter isolated and studied two bacteria which gave nitric oxide from laboratory media. In 1887 Tacke (18) made an extensive quantitative study of the production of nitric oxide, nitrous oxide, and nitrogen from clover, beets, and soil. In one experiment nitric oxide accounted for 26.4% of the nitrogen-containing gas from the fermenting material. Jensen (10) reviewed the subject in 1904 and listed a large number of bacteria and fungi which reduce nitrates and under certain conditions may produce nitric oxide.

Studying reduction of nitrates in experimental plant slurries, Barnett (4) mixed minced grass with potassium nitrate and water and incubated the slurry under both aerobic and anaerobic conditions. About 26% of the added nitrate was converted to nitrite in the aerated mixture and 13% in the unaerated forage. Without added nitrate, nitrite production was only about one tenth of that produced with it. Lactic acid, produced simultaneously with the

nitrite, provided the necessary acid conditions for the release of nitric oxide.

Olson and Moxon (14) studied the production of nitrites from oat hay and red root hay in flask fermentations. Nitrite formation increased for 3 to 4 days and then decreased until, at 10 days, no nitrite was present, presumably because of conversion to molecular nitrogen and nitrous oxide. They did not test for any forms of gaseous nitrogen. The optimum pH for nitrite production was 7 to 8 and none was formed below pH 5.

Production of nitric oxide has been encountered in studies on denitrification processes in soil and in recent years has received considerable attention (12). The principal gases here are molecular nitrogen and nitrous oxide rather than nitric oxide, but the proportions vary with pH, air supply, and concentration of nitrates and organic matter. Wijler and Delwiche (20) reported that nitric oxide made up about 20% of the gas evolved from a soil-alfalfa-nitrate mixture if the pH was held at 4.9 to 5.6 and none at pH 6 or higher. The isotope nitrogen-15 added as labeled sodium nitrate was found in nitrogen, nitrous oxide, and nitric oxide by mass spectrometer analyses.

Najjar and Allen (13) obtained nitric oxide from nitrate and yeast extract at pH 6.7 with extracts of *Pseudomonas stutzeri* bacteria but not with intact cells. Approximately half of the evolved gas consisted of nitric oxide. Baalsrud and Baalsrud (3) reported formation of nitric oxide from nitrate and thiosulfate by *Thiobacillus denitrificans*. The nitric oxide was later slowly reduced to molecular nitrogen by the bacteria.

Nitrogen Oxides in Silos

A systematic study of the occurrence of nitrogen oxides in silage making was undertaken at the College of Agriculture, University of Wisconsin, during the summer of 1956. Air samples from the chutes and neighborhood of five silos (two filled with alfalfa and three with corn) were tested for nitrogen dioxide; in all cases the samples were positive. In two instances enough gas was given off to be clearly visible. Two of these silos were equipped with drain pipes and basins for collecting and measuring the run-off juice. About 30 tons of corn, 0.5 inch cut, was blown into the silo. Silage juice trickled from the drain pipe, and 18 hours later the basin was two thirds full of juice. A steady stream of gas flowed from the pipe at this time and turned a deep brown color on coming in contact with the air. A permanent record of the appearance and movement of the gas was obtained in a colored movie film (copies on file at U.S.D.A. Motion Picture Laboratory, Washington, D. C.). A sample of gas taken from the

basin analyzed about 10% nitrogen dioxide or 100,000 p.p.m. The allowable concentration in industry where workmen may be exposed to the gas—for example, in arc-welding and the manufacture of nitric acid and cellulose films—is 10 to 25 p.p.m.

Oddly enough, a second silo exactly like the first, filled with alternate loads of the same lot of corn, but shredded and cut in ³/₈-inch lengths, gave off no juice and no brown gas. The most probable explanation for the difference in behavior of the two silos is that the drain pipe in the second silo was clogged and the gas was forced to work its way up to the surface and there became diluted with so much air that it was not noticeable. In the other silo the drain pipe furnished an easy outlet for the juice and gas and hence the nitric oxide appeared in a concentration high enough to be visible. Two other silos equipped with drain basins and filled with alfalfa in June 1957, and two others filled with corn in September 1957, gave off juice and easily visible dark brown fumes of nitrogen dioxide.

Laboratory Experiments

Preparation of Silages. Small lots of silage were made in closed bottles equipped for collecting the gas directly in a Van Slyke buret or for absorbing it in potassium hydroxide. A bottle filled with air was placed in the gas train between the forage and alkali, in order to oxidize the nitric oxide to the absorbable nitrogen dioxide. The forages were treated in various ways.

Analytical Methods. The forage was analyzed for moisture, total nitrogen (Kjeldahl), and nitrate nitrogen (reduction by Devarda alloy) (2). The com-

Table I. Mass Analysis of Gas Samples

Mass	Gas	Composition of Gas, Vol. %	
		Alfalfa[a]	Corn[b]
28	N^{14}N^{14}	18.27	29.79
29	N^{14}N^{15}	0.27	0.26
30	N^{14}O^{16}	13.80	12.61
31	N^{15}O^{16}	0.98	0.09
32	O$_2^{16}$	0.36	0.14
40	A	0.14	0.29
44	C^{12}O$_2^{16}$ + N$_2^{14}$O^{16}	65.06	56.00
45	C^{13}O$_2^{16}$ + N^{14}N^{15}O^{16}	0.94	0.67
46	N^{14}O$_2^{16}$	0.29	0.25
		100.11	100.10

[a] Plus 1.7 g. NaN^{15}O$_3$/kg. containing 7.5 atom % excess N^{15}. N$_2$ contained 0.352 atom % excess N^{15} and NO, 6.2 atom % excess N^{15}.
[b] Plus 1.7 g. NaN^{14}O$_3$ and 0.33 g. N^{15} amino acid N/kg. containing 5.2 atom % excess N^{15}. N$_2$ contained 0.079 atom % excess N^{15}.
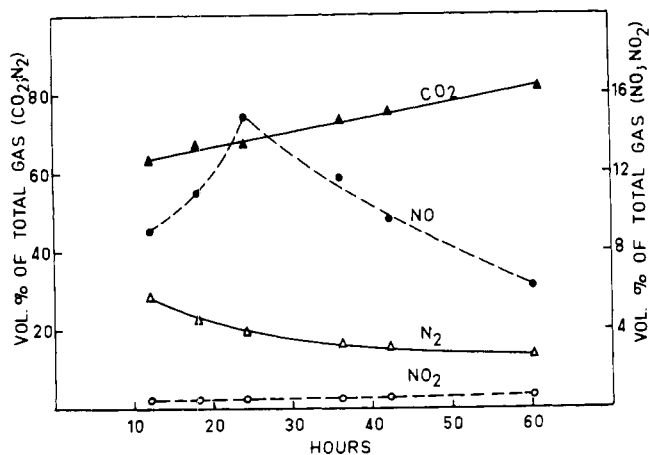
Figure 1. Composition of gas from alfalfa silage, ensiled with N^{15}-labeled nitrate
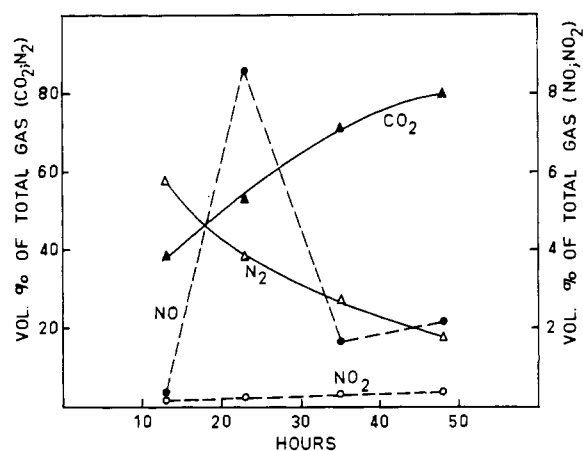

Figure 2. Composition of gas from corn silage. Immature corn ensiled with N^{15}-labeled nitrate

ponents of the gas were determined by mass spectrometer and by the 1-naphthyl-amine–sulfanilic colorimetric method (14). As nitrogen dioxide reacts with the mercury in the mass spectrometer, the figures obtained are probably too low. Carbon dioxide absorbed by the potassium hydroxide solution was determined by addition of excess barium chloride and titration of the precipitated carbonate (2).

Results

Table I gives the detailed data obtained by mass spectrometer on two samples of silage taken at the peak of nitric oxide evolution—i.e., 23 hours. To the alfalfa silage, nitrogen-15–labeled sodium nitrate was added at the time of packing the forage, and to the corn silage a mixture of nitrogen-15–labeled amino acids was added, to throw light on the origin of the different forms of nitrogen in the evolved gas.

Probably the most significant figure is the percentage of nitric oxide in the gas samples: 14.8% in that from alfalfa and 12.70% in that from corn. These data show that nitric oxide is the chief nitrogen oxide given off. It had nearly the same excess of nitrogen-15 isotope, 6.2%, as the added nitrate, 7.5%. Apparently the added nitrate was the chief source of the nitric oxide and only a small amount came from the unlabeled nitrate in the alfalfa. The nitrate nitrogen content of the alfalfa was 0.19 gram per kg.

The small amount of nitrogen dioxide found probably came from nitric oxide that had been oxidized to nitrogen dioxide. Although most of the oxygen trapped in the forage at the time of ensiling was used by the plant tissue and bacteria, 0.1 to 0.2% remained in the samples.

Another interesting feature is the

presence of the nitrogen-15 isotope in the nitrogen gas from both samples. The most logical explanation for its origin is a Van Slyke reaction between nitrous acid and α-amino groups:

$$RCHN^{15}H_2COOH + HNO_2 =$$
$$N^{14}N^{15} + RCHOH \cdot COOH + H_2O$$
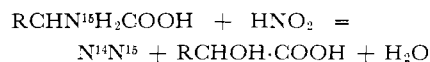
This reaction would account for its presence in both lots of silage, whereas, if it came from the reduction of nitrate, no $N^{14}N^{15}$ gas should have been formed in the corn silage, as this contained labeled amino acids but not labeled nitrate. The atom per cent excess nitrogen-15 was small in both cases, as the nitrogen gas formed in the silage became greatly diluted with the atmospheric nitrogen trapped in the forage at the time of ensiling. As much of the entrained nitrogen was carried out with the fermentation carbon dioxide, the percentage of carbon dioxide gas became correspondingly higher. The argon content of the gas was low as compared with that in air, as would be expected because of its removal with carbon dioxide.

The percentage of gas with mass 45 seems somewhat high to be accounted for as $C^{13}O_2^{16}$. The normal amount of carbon-13 is 1.11 atom %, while the atom per cent of carbon-13 calculated from the data in Table I is 1.42. Recent analyses indicate that also nitrous oxide is formed and if this had been enriched by the added nitrogen-15 to give $N^{14}N^{15}O^{16}$, it would account for the apparently high figure for mass 45. The values for carbon dioxide (mass 44) given in Table I and plotted in the figures are the sum of carbon dioxide and nitrous oxide, as the mass spectrometer does not distinguish between these gases.

Analyses similar to those given in Table I were made on every sample of gas at each period from all lots of experimental silage, but are not given in detail.

Alfalfa Silage

Figure 1 shows the composition of the gas at intervals throughout the fermentation of alfalfa ensiled with nitrogen-15–labeled nitrate. Nitric oxide reached a maximum at 23 hours (14.8%), after which it fell rapidly, but even at 60 hours there was still about 6% present. The nitrogen dioxide content remained less than 0.5% throughout the fermentation, but seemed to rise slowly, perhaps from oxidation of nitric oxide by traces of oxygen in the silage. Carbon dioxide and molecular nitrogen, as would be expected, are in inverse relation. The atmospheric nitrogen trapped in the forage at the time of ensiling was gradually swept out by the carbon dioxide formed by plant respiration and bacterial fermentation.

The volume of carbon dioxide evolved during a given interval was determined and the volume of any other gas was calculated from the average percentages of carbon dioxide and the second gas for that interval.

Sample Calculation. For the period 11 to 18 hours 190 ml. of carbon dioxide were released. The average percentage of carbon dioxide in the gas during this period was 63.3% and the average for nitric oxide was 10%. Then 190/63.3 × 10, or 30, is the number of milliliters of nitric oxide given off.

Such figures are approximately correct and are useful in calculating the volumes of the other gases given off during fermentation. Figures for the first interval are inaccurate, because the average composition of the gas during this time cannot be calculated. The composition of the gas when carbon dioxide began to emerge is unknown, but apparently the carbon dioxide level was appreciable. No gas bubbles were seen passing through the potassium hydroxide for the first 2 or 3 hours. During this time oxygen
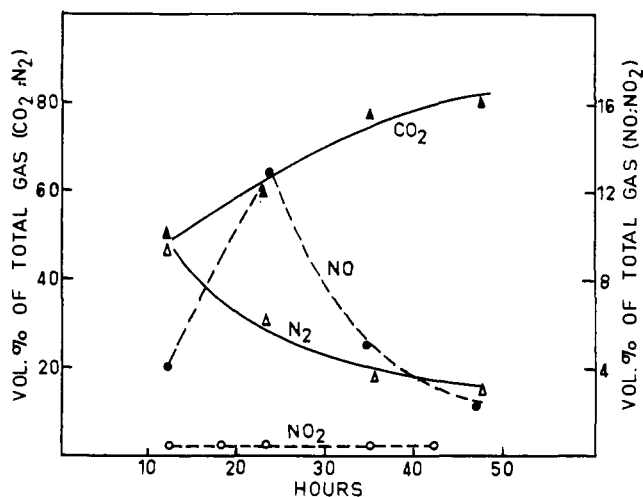
Figure 3. Composition of gas from corn silage. Immature corn ensiled with normal nitrate and N^{15}-labeled amino acids
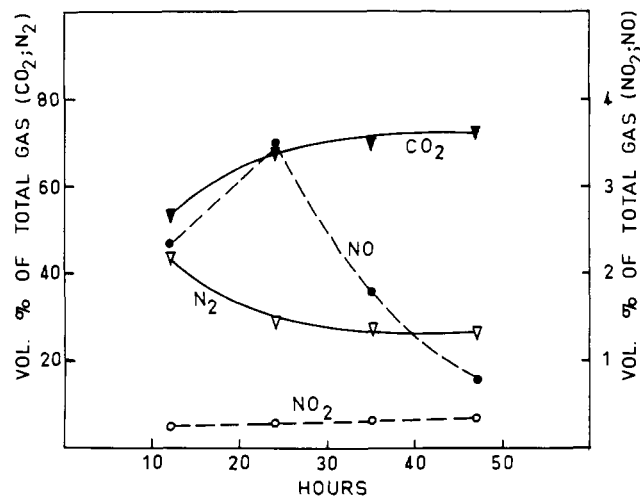

Figure 4. Composition of gas from corn silage. Mature corn ensiled with N^{15}-labeled nitrate

trapped in the silage was being used up and until carbon dioxide production exceeded oxygen consumption there would be no change in gas pressure. At this time the carbon dioxide content of the gas in the silage should be close to the oxygen content of air—i.e., 21%.

Corn Silage. Figures 2 and 3 show the composition of the gas from two lots of immature corn ensiled August 7. To the first lot, nitrogen-15–labeled sodium nitrate was added at the time of ensiling. To the second lot the same quantity of N^{14} nitrate was added, but in addition nitrogen-15–labeled amino acids, equivalent to 0.33 gram of nitrogen, were mixed with the chopped corn. Some nitric oxide was present in the gas from the first lot at 13 hours, and by 23 hours it reached a maximum of 8.6%. It then decreased rapidly to 1.65% at 35 hours.

In the second lot, production of nitric oxide was stimulated by addition of nitrogen-15–labeled amino acids and reached a maximum of 12.2% in 23

hours. A more vigorous fermentation is also indicated here by the carbon dioxide figures, which ranged from 4 to 12% higher than in the corn silage without added amino acids. By the time the peak concentration of nitric oxide was reached, 17.7 ml. of nitric oxide and 181 ml. of carbon dioxide were released from the first lot of corn silage and 25.2 ml. of nitric oxide and 172 ml. of carbon dioxide from the second lot.

Figures 4 and 5 give the data for two corn silages put up at the time when the crop was in the best condition for ensiling. As in the earlier samples, nitrogen-15–labeled sodium nitrate was added to one lot and unlabeled nitrate and nitrogen-15–labeled amino acids were added to the other. Carbon dioxide production was not so fast as with the August silage, probably because the corn was more mature and contained less readily fermentable material. Evolution of nitric oxide from the first sample (Figure 4) began about as promptly as in the August samples, but did not rise to

the high level reached in the earlier silages. The percentages of nitric oxide and nitrogen in the gas from the second samples (Figure 5) were nearly twice those reached in the first sample. The figures for the other components of the gas were about the same for the two silages.

Three other lots of silage were put up and analyzed, but the detailed data on the composition of the evolved gas are not given. However, the volumes of the fermentation gases are given in Table II, with summary data for the five silages reported in Figures 1 to 5. Two lots of sweet corn were ensiled, and here addition of amino acids increased carbon dioxide production about 60% but production of nitric oxide was somewhat less than without amino acids.

The two lots of corn put up on August 7 with nitrate alone may be regarded as duplicate experiments, as they differed only in the presence of excess nitrogen-15 isotope in one case. Carbon dioxide production was about the same in both

Table II. Toxic Gases from Silage Fermentations

| Date (1956)	Forage	Additions[a]	Total Time, Hr.	NO Time to max. concn., hr.	NO Total vol., ml./kg.	NO₂, Ml./Kg.	CO₂, Ml./Kg.
July 3	Alfalfa	NaN¹⁵O₃	71	23	223.7	6.4	1470.0
July 30	Sweet corn	NaN¹⁵O₃	48	48	51.1	1.3	295.0
July 30	Sweet corn	NaN¹⁴O₃ and N¹⁵-labeled amino acids (0.33 g. N)	60	35	42.9	2.3	500.0
Aug. 7	Field corn	NaN¹⁵O₃	52	24	45.9	3.2	712.6
Aug. 7	Field corn	NaN¹⁴O₃ and N¹⁵-labeled amino acids (0.33 g. N)	52	24	101.3	4.3	1032.9
Aug. 7	Field corn	NaN¹⁴O₃	51	24	78.1	3.4	775.3
Sept. 5	Field corn	NaN¹⁵O₃	48	24	25.6	3.6	605.0
Sept. 5	Field corn	NaN¹⁴O₃ and N¹⁵-labeled amino acids (0.33 g. N)	48	12	60.0	3.7	571.3

[a] 1.7 g. NaN¹⁵O₃ or NaN¹⁴O₃ per kg.
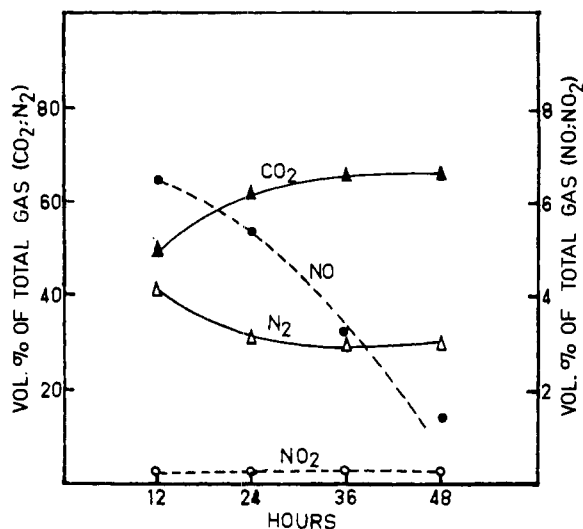
Figure 5. Composition of gas from corn silage. Mature corn ensiled with normal nitrate and N^{15}-labeled amino acids

lots of silage, but nitric oxide production was much greater with the unlabeled nitrate. This difference certainly is not related to the presence of nitrogen-15-labeled nitrate. Reduction of nitrate must depend on the number and kind of bacteria present, their rate of development, and the change in pH of the forage. In an uncontrolled medium great variations are to be expected.

The volume of nitric oxide produced by alfalfa is especially noteworthy—more than twice that produced by any other silage. There was great variation among the silages in the time required to reach the maximum percentage of nitric oxide production and the length of time it continued to be formed thereafter. As the formation of this gas in silage is probably a phase of denitrification, production of nitrous oxide might also be expected. This was looked for in later experiments and definite evidence for its presence was obtained.

Samples of gas from the foregoing silages were also analyzed colorimetrically for nitrogen dioxide at the same time that they were analyzed mass spectrometrically. The data followed the same general pattern as those obtained by mass spectrometric analysis for nitric oxide, but usually were somewhat lower. The figures ranged from 10.3% nitrogen dioxide in the gas at 23 hours for the corn ensiled on August 7 with added nitrate and amino acids to 3.0% for the corn ensiled on September 5 with nitrate alone. The corresponding mass spectrometric values were 12.7 and 3.4% nitric oxide, respectively.

Factors Affecting Nitrogen Dioxide Production

In the following experiments no samples of gas were taken during fermentation, but the potassium hydroxide solution was analyzed for nitrites by the colorimetric method. A difficulty with this method of determination is the absence of a constant ratio of nitrite to nitrate formation in the hydroxide solution. A ratio could not be set up between nitrite determined colorimetrically and total nitrogen obtained by the Devarda alloy method, as the ratios were too erratic to be of value. The data for nitrogen dioxide obtained in the following experiments must therefore be taken as illustrative rather than strictly quantitative.

Table III gives data for several kinds of silage. The experiments with alfalfa with added antiseptics are of particular interest. Chloroform and toluene inhibit the growth of bacteria and in this experiment stopped fermentation, as shown by carbon dioxide production, and consequently no nitrogen dioxide was formed. The frequently used forage preservative, sodium bisulfite, likewise nearly eliminated production of carbon dioxide and nitrogen dioxide. Sterilization and reinoculation of the forage resulted in production of both gases, but in greatly reduced amounts. The several experiments all point to bacteria as the agent responsible for nitric oxide production.

In the soybean experiment the plants were grown without fixed nitrogen and hence obtained their nitrogen by fixation of atmospheric nitrogen by the nodule bacteria with which they were inoculated. The plants probably contained little or no nitrates, and hence nitric oxide production in the silage was practically nil. Addition of nitrates resulted in the formation of a fair amount of nitrogen dioxide.

Addition of nitrate to alfalfa also increased nitrogen dioxide production, but

Table III. Production of Carbon Dioxide and Nitrogen Dioxide in Various Silages as Measured by Potassium Hydroxide Absorption

Forage	Date (1956)	Additions, G./Kg.	Moisture, %	Ensiling Period, Hr.	CO_2, Ml./Kg.	NO_2, Ml./Kg.
Alfalfa[a]	June 29	1 None	70	48	2537	16.7
		2 1.7 g. NaNO₃	70	48	3507	25.0
		3 No. 2 + chloroform and toluene	70	48	0	0
		4 No. 2 + 3.3 g. NaHSO₃	70	48	180	0
		5 Sterilized + 1.7 g. NaNO₃, inoculated with 2% fresh forage	70	48	230	2.1
Soybean	Sept. 27	1 None	70	60	1950	0.14
		2 1.7 g. NaNO₃	70	60	1343	0.68
Corn[a]	Aug. 7	1 None	80	52	710	0.67
		2 1.7 g. NaNO₃	80	52	1065	0.74
	Sept. 5	1 None	74	48	705	1.76
		2 1.7 g. NaNO₃	74	48	642	0.8
		3 No. 2 + chloroform and toluene	74	48	579	0.09
	Sept. 18	1 None	77	60	1367	0.23
		2 1.7 g. NaNO₃	77	60	793	1.25
		3 No. 2 + 1.0 g. NaHSO₃	77	60	546	0.98
Frosted corn	Sept. 27	1 None	71	60	1131	0.63
		2 1.7 g. NaNO₃	71	60	851	0.65
	Oct. 3	1 1.7 g. NaNO₃	55	60	1640	4.46
		2 No. 1 + 3.3 g. NaHSO₃	55	60	980	0.13

[a] Alfalfa contained 0.19 g. NO_3-N/kg. of fresh forage and corn 0.18.

with corn silage the results were irregular. In one experiment more nitrogen dioxide was obtained, in another less, and in two others no effect was apparent. The carbon dioxide data for corn are equally erratic. The October 3 sample with added nitrate produced a large amount of both carbon dioxide and nitrogen dioxide, but in the other experiments there was no regular correlation between production of the two gases.

Addition of chloroform and toluene to the corn sample decreased carbon dioxide production slightly and nitrogen dioxide markedly. Sodium bisulfite reduced formation of both gases but particularly that of nitrogen dioxide if enough were used—1 gram per kg. seemed too little.

Small lots of other plant materials—cabbage, lettuce, and lawn clippings—were ensiled; the evolved gases, tested colorimetrically, all gave a positive test. With nothing added, from 0.4 to 0.8 ml. of nitrogen dioxide per kg. was released. With added nitrate the quantity of nitrogen dioxide from cabbage rose from 0.6 to 1.6 ml. per kg. Addition of 2.5% sodium chloride (as in the making of sauerkraut) reduced nitrogen dioxide production in one experiment but had no effect in another.

Summary

Five silos were tested for the production of nitrogen dioxide during the summer and fall of 1956. All gave positive tests. Three of the silos were equipped with drainage basins for collecting the expressed silage juice and in two cases the basins became filled with the brown fumes. A good colored movie film of the fumes was obtained. A sample of the gas in the basin analyzed about 10% nitrogen dioxide.

Gases from eight lots of experimental silage to which nitrogen-15-labeled nitrate or amino acids were added were analyzed by means of the mass spectrometer and found to contain from 3.4 to 14.8% nitric oxide and usually less than 0.5% nitrogen dioxide. The atom per cent excess nitrogen-15 was about the same in the nitric oxide as in the added nitrate, indicating that most of it came from the latter. As expected, the label in nitric oxide was about 10 times as high when $NaN^{15}O_3$ was added to the forage as when nitrogen-15-labeled amino acids were ensiled with the plant material. The percentage of $N^{14}N^{15}$ was about the same in the gases from the two silages, hence its formation is attributed to a Van Slyke reaction between nitrous acid and α-amino nitrogen.

Other lots of silage were made from several kinds of forage with various treatments. Added sodium nitrate usually increased production of nitrogen dioxide. Chloroform and toluene prevented formation of gas, and sodium bisulfite reduced the amount. Sterilization and reinoculation of the forage gave reduced but definite amounts of nitrogen dioxide. These data indicate that bacteria and not plant enzymes are responsible for the formation of the nitrogen oxides.

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FEEDSTUFFS ANTIOXIDANTS

Stabilization of Alfalfa Carotenoids with N,N'-Diaryl-alpha,omega-diaminoalkanes

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Several N,N'-diaryl-α,ω-diaminoalkanes were synthesized and tested as antioxidants for the carotenoids of alfalfa. Increasing the length of the aliphatic portion of the molecule improved the antioxidant activity of the compounds, indicating lipide solubility to be an important factor. Compounds having methoxy groups in the para positions were more active than the corresponding methyl-substituted compounds, which in turn were more active than the unsubstituted substances.

THE ABILITY of various organic compounds to stabilize the carotene of dehydrated alfalfa meal has been re-

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ported (2, 8, 11, 14). The most promising appeared to be DPPD (N,N'-diphenyl-p-phenylenediamine) and Santoquin (6-ethoxy-2,2,4-trimethyl-1,2-dihydroquinoline). However, the Food and Drug Administration recently re-

quested that addition of DPPD to feedstuffs be discontinued (10), because it caused toxic effects with certain test animals (12). Santoquin has been approved for use in poultry feeds only. Hence there still is need for an all-pur-