CONTROLLED ATMOSPHERE STUDIES

The purpose of the controlled atmosphere studies was to determine the effects of carbon dioxide (CO_2), oxygen (O_2), and ethylene (C_2H_4) levels on Merion Kentucky bluegrass sod. The sod heating box experiments gave considerable information on the levels of CO_2, O_2, and ethylene in relation to cultural treatments that occur during storage under simulated shipping conditions, but no information on the direct effects of these gases on sod injury. Further information on the physiological mechanisms involved in sod heating and damage was obtained by storing sod under controlled conditions in atmospheres containing 0, 9, 18, and 27% CO_2, and 0, 2, 4, 8, and 16% O_2, and 0, 2, 4, and 8 ppm ethylene in factorial combinations.

Methods and Materials for Controlled Atmosphere (CA) Studies

The following paragraphs describe the methods and materials used in these studies.

Gas mixtures.--Prepared gas mixtures were purchased from The Matheson Company, Inc., at Joliet, Illinois. The gas mixtures ordered and the gas content of the mixtures received are presented in Table 38. The gas content was

Table 38. Prepared gas mixtures ordered and actual analysis (in parentheses) of gas mixtures received and used

CO_2 - O_2 - C_2H_4[a] (%) (%) (ppm)	CO_2 - O_2 - C_2H_4 (%) (%) (ppm)	CO_2 - O_2 - C_2H_4 (%) (%) (ppm)	CO_2 - O_2 - C_2H_4 (%) (%) (ppm)
0 - 0 - 0 (0.0 - 0.0 - 0.0)		0 - 0 - 4 (0.1 - 0.9 - 4.5)	
18 - 0 - 0 (17.6 - 0.2 - 0.0)			
0 - 2 - 0 (0.1 - 2.0 - 0.0)	0 - 2 - 2 (0.1 - 2.3 - 2.3)	0 - 2 - 4 (0.1 - 2.2 - 4.2)	0 - 2 - 8 (0.1 - 2.3 - 9.1)
9 - 2 - 0 (8.6 - 2.1 - 0.0)			
18 - 2 - 0 (17.7 - 2.3 - 0.0)	18 - 2 - 2 (17.6 - 2.3 - 2.2)	18 - 2 - 4 (17.8 - 2.0 - 4.0)	18 - 2 - 8 (17.9 - 2.1 - 7.9)
27 - 2 - 0 (28.4 - 2.8 - 0.0)			
0 - 4 0 (0.1 - 4.6 - 0.0)	0 - 4 - 2 ((0.1 - 4.5 - 2.3)	0 - 4 - 4 (0.1 - 4.6 - 4.7)	0 - 4 - 8 (0.1 - 4.6 - 9.0)
18 - 4 - 0 (16.9 - 4.6 - 0.0)	18 - 4 - 2 (16.9 - 4.5 - 2.0)	18 - 4 - 4 (16.8 - 4.6 - 4.1)	18 - 4 - 8 (17.4 - 4.6 - 8.1)
0 - 8 - 0 (0.1 - 7.8 - 0.0)			
9 - 8 - 0 (8.6 - 8.7 - 0.0)			
18 - 8 - 0 (16.6 - 8.1 - 0.0)		18 - 8 - 4 (16.9 - 9.0 - 4.1)	
27 - 8 - 0 (27.6 - 9.0 - 0.0)			
0 - 16 - 0 (0.0 - 16.1 - 0.0)			0 - 16 - 8 (0.1 - 16.1 - 8.3)
18 - 16 - 0 (16.6 - 15.6 - 0.0)			

[a]The remainder of each mixture was nitrogen gas.

determined by analyzing small samples with the Vapor Fractometer and the Varian Aerograph gas chromatographs. The cylinders were the 1A size (9 inches in diameter x 52 inches in length) and contained approximately 220 cu ft of gas. The selection of gas mixtures was based on consideration of the questions to be answered by the research and statistical requirements.

General methods for CA studies.--The overall apparatus for the controlled atmosphere studies is shown in Figure 8. A two-stage pressure regulator was connected to each gas cylinder. The outlet from each regulator was equipped with a needle-type metering valve. Tygon tubing, having a 0.25 inch inside diameter was connected to the CA chambers. Three of the chambers were connected in series to each cylinder. Five 6 inch diameter sod pieces were placed in each chamber at the beginning of each gas experiment. The gas flow was measured with a small plastic flowmeter and adjusted with the metering valve. One sod piece was removed from each chamber every 24 hours and transplanted to a sand-filled pot in the greenhouse. A dark growth chamber was used to maintain a constant 86° F temperature.

CA chambers.--The CA chambers were made from 8 qt polyethylene buckets (Figure 9). Two quarts of cement were poured into each bucket to reduce the volume of the chambers and therefore the amount of gas required. The buckets were completely lined with Visqueen pressure tape to seal against ethylene loss. A small rack was built and used to hold the

Figure 8. Overall apparatus for controlled atmosphere experiments showing gas delivery and flow metering system.

Figure 9. Controlled atmosphere bucket chambers. The lid, gas inlet tube, rack, and arrangement of sod pieces are shown.

sod pieces 0.5 inch above the bottom of the chamber. The arrangement of the 5 sod pieces inside the chambers was as follows: 4 were set on edge around the wall of the chamber with the grass facing inward and the fifth was set on edge in the middle. The chamber lid was made from a fruit storage can lid which fit snugly inside the top of the bucket. Two 0.25 inch diameter by 2 inch long pipes were inserted through the lid and brazed into place. The inlet pipe had a 10 inch length of tubing attached. The end of this tubing was placed under the rack as the chambers were closed. This was done to increase the uniformity of gas flow around the sod pieces. The lids were sealed with pressure tape.

Gas flow rates.--The gas flow rate was set at 0.6 cu ft per hour. Measurements showed that each chamber contained about 0.15 cu ft of free space when the 5 sod pieces were in place. The atmosphere within each chamber was changed 4 times per hour. The gas flow rate was measured by attaching a small flowmeter to the outlet of the third chamber in each series. The needle valve was used to adjust the flow rate. The flow rate was set daily after removing the sod piece. It was checked and adjusted, if necessary, at about 8 hour intervals.

The composition of the gas in each chamber was checked each day just before removing the sod piece. The inlet and outlet pipes were capped with sleeve-type rubber stoppers. A 10 cc gas sample was withdrawn with a needle syringe and analyzed. The percent carbon dioxide generally increased a few tenths of a percent between chambers 1 and 3 of the series. The percent oxygen decreased slightly over the series. Ethylene levels decreased somewhat, especially for gas mixtures containing 8 ppm ethylene. Nevertheless, the 0.60 cu ft per hour flow rate which changed the atmosphere in the chambers 4 times per hour was satisfactory for these experiments.

Sod source.--The Merion Kentucky bluegrass sod was obtained from the source described for the August 24, 1969 experiment. It was high in quality, two years old, and had received a total of 340 lb/A of nitrogen during its production period. The sod was harvested between September 22 and October 21, 1969. Some hardening of the sod was probably occurring during this period of time.

Gas experiments.--Five gas experiments were performed. At the beginning of each gas experiment the sod was cut at a 0.75 inch depth and into 6 inch diameter pieces. Five sod pieces were arranged over the racks inside each chamber and the lids were sealed. Six gas mixtures were selected. Each cylinder was connected with tubing to a series of 3 chambers. One sod piece was removed from each chamber every 24 hours. The sod pieces were transplanted to pots filled with sand and placed under the mist irrigation system in the greenhouse. Visual estimates of percent leaf kill were made 2 days after transplanting.

Visual estimates of percent leaf cover were made 30 days after transplanting. Then the root organic matter production was determined using the methods described earlier. The initiation dates, temperature and percent moisture of the sod, and the gas mixtures used in each experiment are presented in Table 39.

Table 39. Date initiated, temperature and percent moisture of the sod, and the gas mixture used in each gas experiment

Date	Temp. (°F)	Moisture (%)	Gas Mixtures Used CO_2 - O_2 - C_2H_4 (%) (%) (ppm)	Gas Mixtures Used CO_2 - O_2 - C_2H_4 (%) (%) (ppm)
9/22	69	65	18 - 4 - 0	18 - 4 - 2
			18 - 4 - 4	18 - 4 - 8
			18 - 8 - 0	18 - 8 - 4
9/27	56	67	0 - 4 - 0	0 - 4 - 2
			0 - 4 - 4	0 - 4 - 8
			0 - 16 - 0	0 - 16 - 8
10/8	56	71	0 - 2 - 0	0 - 2 - 2
			0 - 2 - 4	0 - 2 - 8
			0 - 0 - 0	0 - 0 - 4
10/14	52	70	18 - 2 - 0	18 - 2 - 2
			18 - 2 - 4	18 - 2 - 8
			9 - 2 - 0	27 - 2 - 0
10/21	45	72	0 - 0 - 0	0 - 8 - 0
			9 - 8 - 0	18 - 0 - 0
			18 - 16 - 0	27 - 8 - 0

Statistical analysis.--To gain the most information about the sod response to gas levels, the results from selected gas mixtures of several gas experiments were pooled together in various ways and analyzed as factorial analysis of variance (AOV) experiments. Thus, any differences in response which may have resulted from difference in sod temperature, moisture percentage or degree of hardening were confounded with responses to gas mixtures. Matching sets of gas mixtures were included in each gas experiment to minimize this disadvantage. The data from the sod contained in each 3-chamber series were treated as 3 replications. This eliminated response differences resulting from changes in gas levels over the 3 chambers from the error term. The main effect means for replications were never different.

Results of Controlled Atmospheres Studies

The statistical analysis for one way of grouping the data gathered from the gas experiments will be discussed in each of the following sections.

CA statistical comparison I.--A summary of gas mixtures whose effects were compared in this statistical analysis are presented in Table 40.

The main effect means for root production in relation to carbon dioxide, oxygen, and ethylene levels after storage under controlled atmospheres are presented in Table 41. The mean root production in relation to storage

Table 40. Gas mixtures whose effects were compared by factorial AOV in "CA statistical comparison I"

CO_2 - O_2 - C_2H_4 (%) (%) (ppm)	CO_2 - O_2 - C_2H_4 (%) (%) (ppm)	CO_2 - O_2 - C_2H_4 (%) (%) (ppm)	CO_2 - O_2 - C_2H_4 (%) (%) (ppm)
0 - 2 - 0	0 - 2 - 2	0 - 2 - 4	0 - 2 - 8
0 - 4 - 0	0 - 4 - 2	0 - 4 - 4	0 - 4 - 8
18 - 2 - 0	18 - 2 - 2	18 - 2 - 4	18 - 2 - 8
18 - 4 - 0	18 - 4 - 2	18 - 4 - 4	18 - 4 - 8

time was relatively constant. When comparing the response of sod stored under 0 and 18% CO_2, more root production occurred in response to the 18% CO_2 level after 24 hours of storage while the 0% CO_2 level resulted in more root production after 96 hours of storage. The 4% O_2 level resulted in greater root production after 24, 48, and 72 hours of CA storage, but more root production resulted from the 2% O_2 level after 120 hours of CA treatment. Ethylene levels of 4 and 8 ppm depressed root production as compared to 0 and 2 ppm of ethylene. These results for ethylene levels were significant after 24, 48, and 120 hours of CA storage. It was clear that ethylene levels of 4 ppm were harmful to sod rooting, but apparently the 8 ppm level of ethylene did not result in greater damage. No leaf injury or differences in percent leaf cover occurred.

Table 41. Main effect means for root production data from Merion Kentucky bluegrass sod stored under the controlled atmospheres listed in Table 40 for 5 days

Hours of Storage	Mean	Root Organic Matter Production (mg/pot)							
		% CO_2		% O_2		ppm C_2H_4			
		0	18	2	4	0	2	4	8
24	79	52	107***	45	114***	99a	105a	55b	59b*
48	66	62	69	52	80***	77a	93a	45b	48b***
72	75	71	79	62	88**	81ab	100a	62bc	56c**
96	68	84	51***	65	70	71ab	87a	48c	64bc**
120	81	79	82	92	69*	92a	107a	64b	60b**

*, **, ***Differences between main effect means are significant at the .05, .01, and .001 level of probability, respectively, for designated hours of storage. Main effect means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the .05 level.

Significant carbon dioxide x oxygen interactions for root production occurred after 24, 72, and 120 hours of CA storage (Table 42). The treatment combinations of 18% CO_2 and 4% O_2 stimulated root production significantly after 24 hours of CA storage. In the presence of 2% O_2, the 18% CO_2 level significantly decreased root production while 18% CO_2 increased root production after 72 hours at the 4% O_2 level. Also, 4% O_2 stimulated root production when 18% CO_2 was present after 72 hours. In the presence of 18% CO_2, exposure to 4% O_2 for 120 hours of storage decreased root production. These results suggest that the combination of 18% CO_2 and 4% O_2 stimulates root production for up to 72 hours

Table 42. Significant carbon dioxide x oxygen concentration interactions for root production (mg/pot) of Merion Kentucky bluegrass sod stored under the controlled atmospheres listed in Table 40 for 5 days

Hours of Storage	% CO_2	% O_2		Simple Effects
		2	4	
24	0	49	55	6
	18	41	173	132**
	Simple Effects	-8	118**	
72	0	77	65	-12
	18	46	112	66**
	Simple Effects	-31**	47**	
120	0	81	77	-4
	18	104	61	-43**
	Simple Effects	23	-16	

*, **Differences are greater than LSD at the .05 and .01 level, respectively.

of CA storage, but that the presence of 4% O_2 resulted in more rapid depletion of carbohydrate reserves and decreased root production after 120 hours of CA storage.

A significant oxygen x ethylene interaction for root production occurred after 48 hours of CA storage (Table 43). In the presence of 0 and 2 ppm ethylene, the 4% O_2 level increased root production significantly. The 4 and 8 ppm ethylene levels decreased root production in the presence of 4% O_2. Oxygen simulated root production at low ethylene levels while high ethylene levels decreased root production when adequate oxygen was available. The trend in the oxygen x ethylene interaction was similar to the above after 24, 72, and 96 hours of CA storage.

<u>CA statistical comparison II</u>.--A list of gas mixtures whose effects were compared in this statistical analysis are presented in Table 44.

The main effect means for root production in relation to 2, 4, and 8% O_2 and 0 and 4 ppm ethylene after storage in controlled atmospheres are presented in Table 45. More root production occurred in response to 4 and 8% O_2 after 24 hours of storage. However, the 4 and 8% O_2 levels resulted in decreased root production after 120 hours of storage. Four ppm ethylene depressed root production significantly after 24 and 120 hours of storage. No leaf injury resulted from these treatments.

Table 43. Significant oxygen x ethylene concentration interaction for root production of Merion Kentucky bluegrass sod stored under the controlled atmospheres listed in Table 40

Measurement	Hours of Storage	% O_2	ppm Ethylene 0 a_1	2 a_2	4 a_3	8 a_4	Simple Effects a_2-a_1	a_3-a_1	a_4-a_1	a_3-a_2	a_4-a_2	a_4-a_3
Root organic matter (mg/pot)	48	2	49	67	38	53	18	-11	4	-29*	-14	15
		4	105	119	52	43	14	-53**	-62**	-67**	-76**	-9
Simple Effects			56**	52**	14	-10						

*, **Differences are greater than LSD at the .05 and .01 level, respectively.

Table 44. Gas mixtures whose effects were compared by factorial AOV in "CA statistical comparison II"

CO_2 - O_2 - C_2H_4 (%) (%) (ppm)	CO_2 - O_2 - C_2H_4 (%) (%) (ppm)
18 - 2 - 0	18 - 2 - 4
18 - 4 - 0	18 - 4 - 4
18 - 8 - 0	18 - 8 - 4

Table 45. Main effect means for root production (mg/pot) for Merion Kentucky bluegrass sod stored in the controlled atmospheres listed in Table 44 for 5 days

Hours of Storage	Mean	% O_2			ppm C_2H_4	
		2	4	8	0	4
24	129	41	181	165**	169	89*
48	78	59	86	90	84	72
72	97	46	110	134	98	95
96	57	56	55	61	66	48
120	77	108	66	58*	92	62*

*, **Differences between main effect means are significant at the .05 and .01 level of probability, respectively, for designated hours of storage.

CA statistical comparison III.--Gas mixtures whose effects were compared in this statistical analysis are presented in Table 46.

The main effect means for root production in relation to oxygen and ethylene levels are presented in Table 47. More root production occurred at 0% O_2 than at the 2% O_2 level after 24 hours of storage. This was unexpected since the 0% O_2 treatment caused 17% leaf kill after 24 hours of storage. The 0% O_2 level resulted in significantly less root production than 4% O_2 after 48 hours. The 2 and 4% O_2 levels gave more root production than 0% O_2 after 72, 96 and 120 hours of storage. In the absence of oxygen, the turfgrass plants died rapidly, but the presence of only 2% O_2 was sufficient to sustain the metabolism of the plants for 120 hours. The 4 ppm ethylene level depressed root production significantly after 48, 72, and 96 hours.

For the zero O_2 level the leaf kill over time was as follows:

24	48	72	96	120	Hours
17	93	98	100	100	% leaf kill

No leaf kill occurred when oxygen was present until after 120 hours. Then 10% leaf kill occurred in response to the 4% O_2 level. This suggests that, in the presence of 4% O_2, the respiration rate was great enough to deplete carbohydrate reserves and resulted in some leaf injury after 120 hours of storage.

Table 46. Gas mixtures whose effects were compared by factorial AOV in "CA statistical comparison III"

CO_2 (%)		O_2 (%)		C_2H_4 (ppm)	CO_2 (%)		O_2 (%)		C_2H_4 (ppm)
0	-	0	-	0	0	-	0	-	4
0	-	2	-	0	0	-	2	-	4
0	-	4	-	0	0	-	4	-	4

Table 47. Main effect means for root production (mg/pot) for Merion Kentucky bluegrass sod stored in the controlled atmospheres listed in Table 46 for 5 days

Hours of Storage	Mean	% O_2			ppm C_2H_4	
		0	2	4	0	4
24	50	66a	39b	48ab*	55	46
48	43	17b	41ab	72a**	56	31*
72	48	12b	72a	59a***	63	32***
96	45	3b	60a	72a***	56	35**
120	50	3b	80a	67a***	57	43

*, **, ***Differences between main effect means are significant at the .05, .01, and .001 level of probability, respectively, for designated hours of storage. Main effect means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% level.

<u>CA statistical comparison IV</u>.--A summary of the gas mixtures whose effects were compared in this statistical analysis are presented in Table 48.

Table 48. Gas mixtures whose effects were compared by factorial AOV in "CA statistical comparison IV"

CO_2 (%)	-	O_2 (%)	-	C_2H_4 (ppm)	CO_2 (%)	-	O_2 (%)	-	C_2H_4 (ppm)
0	-	0	-	0	18	-	0	-	0
0	-	2	-	0	18	-	2	-	0
0	-	4	-	0	18	-	4	-	0
0	-	8	-	0	18	-	8	-	0
0	-	16	-	0	18	-	16	-	0

The main effect means for root production and percent leaf kill in relation to carbon dioxide and oxygen levels are presented in Table 49. The 18% CO_2 level resulted in significantly greater root production after 24 hours of storage and significantly less after 96 hours. When oxygen was absent, low root production occurred after 24 hours and no root production occurred after 48, 72, 96, and 120 hours of CA storage. The highest numerical values for root production occurred at the 4, 4, 8, 4, and 2% O_2 levels for 24, 48, 72, 96, and 120 hours, respectively.

Table 49. Main effect means for root production and percent leaf kill for Merion Kentucky bluegrass sod stored in the controlled atmospheres listed in Table 48 for 5 days

Measurement	Hours of Storage	Mean	% CO_2		% O_2				
			0	18	0	2	4	8	16
Root organic matter (mg/pot)	24	92	55	128**	37c	57bc	158a	137ab	68abc**
	48	68	59	77	0c	55b	105a	92ab	88ab***
	72	59	55	64	0c	71ab	94ab	102a	30bc***
	96	54	64	45*	0b	65a	83a	75a	49a***
	120	62	62	62	0c	107a	83ab	75ab	47b***
% Leaf kill	24	8	8	8	40	0	0	0	0***
	48	20	20	20	100	0	0	0	0***
	72	20	20	20	100	0	0	0	0***
	96	23	24	22	100	0	0	5	10***
	120	26	31	21	100	0	5	8	18***

*, **, ***Differences between main effect means are significant at the .05, .01, and .001 level of probability, respectively, for designated hours of storage. Main effect means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% level.

Oxygen levels of 4 to 8% gave the best root production results for sod storage time of up to 96 hours. A 2% O_2 level was best when the sod was stored 120 hours.

Forty percent leaf kill occurred after 24 hours of CA storage without oxygen. The leaf kill was 100% for longer periods of storage without oxygen. No leaf kill occurred in the presence of oxygen until after 96 hours of storage. The leaf kill was 5 and 10% for 8 and 16% O_2, respectively, after 96 hours. Leaf kill was 5, 8, and 18% for 4, 8, and 16% O_2, respectively, after 120 hours. Carbon dioxide levels did not affect leaf kill.

Significant carbon dioxide x oxygen concentration interactions for root production occurred after 24, 48, 72, and 120 hours of CA storage (Table 50). The pattern of response shows increased root production in response to 4 and 8% O_2 in the presence of 18% CO_2 for 24, 48, and 72 hours (except 16% O_2 after 48 hours). The 18% CO_2 and 2% O_2 treatment combinations gave the most root production after 120 hours. These interactions also indicate a shift in optimum oxygen levels for sod storage from 4 to 8% O_2 to a 2% O_2 level when the length of CA storage increases beyond 72 hours.

Table 50. Significant (.05 level) carbon dioxide x oxygen interactions for root production (mg/pot) of Merion Kentucky bluegrass sod stored in the controlled atmospheres listed in Table 48 for 5 days

Hours of Storage	% CO_2	% O_2 0	2	4	8	16
24	0	41	51	57	90	38
	18	33	63	258	184	99
48	0	0	46	97	107	44
	18	0	64	113	76	133
72	0	0	84	87	69	34
	18	0	59	101	135	26
120	0	0	88	79	86	59
	18	0	127	87	63	36

CA statistical comparison V.--A summary of gas mixtures whose effects were compared in this statistical analysis are presented in Table 51.

Table 51. Gas mixtures whose effects were compared by factorial AOV in "CA statistical comparison V"

CO_2 (%)	O_2 (%)	C_2H_4 (ppm)	CO_2 (%)	O_2 (%)	C_2H_4 (ppm)
0	2	0	0	8	0
9	2	0	9	8	0
18	2	0	18	8	0
27	2	0	27	8	0

The main effect means for root production in relation to carbon dioxide and oxygen levels are presented in Table 52. Significant differences in response to carbon dioxide levels occurred after 24, 72, and 120 hours of CA storage. More root production occurred in response to 18% CO_2 than to the other levels after 24 hours. The 27% CO_2 level resulted in less root production than the 0 and 18% CO_2 levels after 72 hours. The root production for 27% CO_2 was significantly less than for the other carbon dioxide levels after 120 hours of storage. The 8% O_2 level resulted in significantly more root production than 2% O_2 after 24 and 48 hours. The 2% O_2 level resulted in significantly more root production after 120 hours of CA storage.

Table 52. Main effect means for root production (mg/pot) of Merion Kentucky bluegrass sod stored in the controlled atmospheres listed in Table 51 for 5 days

Hours of Storage	Mean	% CO_2				% O_2	
		0	9	18	27	2	8
24	12	71b	61bc	123a	35c***	39	106***
48	71	77	68	70	71	50	93**
72	64	76bc	45bc	97a	38c	59	69
96	62	74	47	66	59	67	56
120	84	87a	92a	95a	60b*	101	66***

*, **, ***Differences between main effect means are significant at the .05, .01, and .001 level of probability, respectively, for designated hours of storage. Main effect means followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% level.

Significant oxygen x carbon dioxide concentration interactions for root production occurred after 24, 72, 96, and 120 hours of CA storage (Table 53). The 8% O_2 and 18% CO_2 treatment combination resulted in the greatest root production after 24 and 72 hours. The 27% CO_2 and 2% O_2 treatment combination increased root production while the 27% CO_2 and 8% O_2 treatment combination decreased root production after 96 hours. The 9 and 18% CO_2 with 2% O_2 increased root production after 120 hours.

Table 53. Significant (.05 level) oxygen x carbon dioxide concentration interactions for root production (mg/pot) of Merion Kentucky bluegrass sod stored in the controlled atmospheres listed in Table 51 for 5 days

Hours of Storage	% O_2	% CO_2			
		0	9	18	27
24	2	51	28	63	14
	8	90	93	184	56
72	2	84	54	58	38
	8	69	35	135	38
96	2	66	50	64	89
	8	82	44	68	30
120	2	88	113	126	72
	8	86	72	63	43

CA temperature experiment.--The response of sod to 8 days of CA storage at 104 and 83° F was studied in this experiment. Two CA chambers were placed in the growth chamber (104° F) and 2 were set on the floor (83 ± 1° F) for each gas mixture used. Temperatures were measured twice a day. The 4 chambers were connected in series. The gas flow rate was set at 0.3 cu ft per hour. The general methods were the same as described in the Methods and Materials for Controlled Atmosphere (CA) Studies section.

The averages for percent leaf kill and root production data in relation to temperature and the gas mixtures used are presented in Table 54. The duration of leaf survival and amount of root production was surprisingly high for sod stored in pure nitrogen gas (0-0-0) at 83° F. Storage in the 18-16-0 gas mixture resulted in the lowest percent leaf kill and the highest root production, especially after 6 and 8 days at 83° F. The results for the sod stored at 83° F were similar to those obtained in the previous CA studies. Very high levels of leaf kill occurred after only 2 days of storage at 104° F. The sod was completely killed after 4 or more days of 104° F storage. These results show a definite positive relationship between sod temperature and injury.

Total available carbohydrates (TAC) for the CA temperature experiment.--The TAC levels for the sod used in the CA temperature experiment were determined initially and after 8 days of storage. The final determination was made on grass stems collected from 1 chamber for each gas

Table 54. Percent leaf kill and root production in relation to temperature of Merion Kentucky bluegrass sod stored in the gas mixtures listed for 8 days

Gas Mixture		Percent Leaf Kill				Root Production (mg/pot)			
		Days of Storage				Days of Storage			
CO_2-O_2-C_2H_4 (%) (%) (ppm)	Temp. (^oF)	2	4	6	8	2	4	6	8
0 - 0 - 0	104	100	100	100	100	0	0	0	0
	83	0	45	92	80	95	53	38	0
18 - 2 - 8	104	98	100	100	100	0	0	0	0
	83	0	20	55	65	106	112	78	46
27 - 2 - 0	104	92	100	100	100	6	0	0	0
	83	0	12	75	90	82	68	49	11
18 -16 - 0	104	70	95	100	100	22	8	0	0
	83	0	0	30	20	78	147	164	154

mixture and temperature. The TAC determinations were made using the methods described in the October 24, 1969, sod heating box experiment section.

The TAC data in relation to temperature, leaf kill, and root production are presented in Table 55. The TAC of the sod was 32.9% at the beginning of the experiment.

The sod stored in nitrogen gas (0-0-0) had 18.1 and 17.6% TAC for 104 and 83° F storage temperatures, respectively. The sod stored at 104° F was dead after 2 days while 8% of the plants for sod stored at 83° F survived 6 days of storage (see Table 54). Nevertheless, the TAC levels were about the same for sod stored under the two temperature regimes.

The sod stored in the 18-2-8 atmosphere had 16.9 and 18.6% TAC for 104 and 83° F storage temperatures, respectively. The sod stored at 104° F was dead after 2 days. The sod stored at 83° F had 60% leaf kill and a moderate level of root production after 8 days of storage.

Initially, the sod stored in the 27-2-0 atmosphere had 23.2 and 18.4% TAC for 104 and 83° F temperatures, respectively. The sod stored at 104° F was dead after 4 days of storage and still retained 23.2% TAC. The sod stored at 83° F had 90% leaf kill and a low level of root production after 8 days of storage.

The sod stored in the 18-16-0 atmosphere had 28.8 and 24.0% TAC for 104 and 83° F storage temperatures, respectively. The sod stored in this atmosphere had survived

Table 55. Percent total available carbohydrate (TAC) in relation to temperature, percent leaf kill, and root production of Merion Kentucky bluegrass sod harvested on October 29, 1969, and stored for 8 days in the gas mixtures listed

Gas Mixtures CO_2-O_2-C_2H_4 (%) (%) (ppm)	Temp. (^oF)	% TAC[a]	% Leaf Kill	Root Production (mg/pot)
0 - 0 - 0	104	18.1	100	0
	83	17.6	70	0
18 - 2 - 8	104	16.9	100	0
	83	18.6	60	73
27 - 2 - 0	104	23.2	100	0
	83	18.4	90	8
18 - 16 - 0	104	28.8	100	0
	83	24.0	20	180

[a]The sod had 32.9% TAC at the beginning of the experiment.

the 104° F temperature better than sod stored in the other atmospheres. The sod at 83° F had only 20% leaf kill and high root production after 8 days of storage. A higher respiration rate and more rapid depletion of TAC was expected in the presence of 16% O_2, but this did not occur. The sod retained more than 75% of its original TAC level and had the best survival.

Apparently the high temperature (104° F) resulted in direct injury to the sod. No evidence for the depletion of carbohydrates to some critically low level before sod injury occurred was found in this experiment.

load. The major part of the load was 4 rolls wide and 6 rolls or tiers high (35 inches). This was held in place by topping the load with a section that was 3 rolls wide and 3 rolls deep. Finally, a canvas tarp was tied in place over the top of the load.

Ventilation tubes and thermocouple wires were inserted into the load while the sod was being loaded. The ventilation tubes consisted of 4 inch diameter by 8 feet long sections of polyvinylchloride drain tile, which had 0.5 inch diameter holes drilled on 1.2 inch centers. An elbow, attached to each tube, pointed forward to catch air during transport. Four ventilation tubes were placed on top of the bottom tier of sod rolls. The tubes were spaced at 5 foot intervals in the load. Thermocouples were inserted into the center of the 2 sod rolls located in the central 2 rows and immediately behind the ventilation tubes in the second tier. Thermocouples were similarly placed in sod rolls located 1 roll further away from the ventilation tube. Thermocouples were also placed in sod rolls in the central 2 rows of the first through the sixth tiers from the bottom of the load. Temperature was measured with a Leeds and Northrup portable potentiometer and 18 gauge copper-constantan thermocouples.

Results and discussion.--The main effect means for temperature changes in relation to distance from the ventilation tubes are presented in Table 56. The initial temperature was 66^{o} F; this was low for July 10. June of 1968 was cool with very high rainfall. One inch of rain fell on

Table 56. Temperature (^{o}F) changes in relation to distance from ventilation tubes inserted across a semi-trailer load of commercial sod during 20 hours of storage on the load for Merion Kentucky bluegrass sod harvested on July 10, 1968

Hours on Load	Mean	Distance Behind Ventilator (in)		Air Temperature
		6	18	
0	66.2	66.2	66.1	60
6	69.3	70.0	68.6	76
12	70.6	70.5	70.6	67
20	72.0	71.9	72.2	63

July 9. These two facts contributed to the low initial temperature. The temperature increased about 6^{o} F during the 20 hours that sod was in the load. The initial temperature measurements were taken immediately after the sod loading was completed. The 12 hour temperature measurements were taken just after the sod load arrived in Cleveland after 4 hours of highway travel. The temperature within sod rolls adjacent (6 inches) to the ventilation tubes was the same as that 18 inches away. This clearly shows that the ventilation tubes were not effective in reducing temperature.

The main effect means for temperature changes in relation to distance from the bottom of the load are presented in Table 57. The distance from the bottom of the sod load did not affect temperature. The sod was not injured during shipment.

Table 57. Temperature (^{o}F) changes in relation to distance from the bottom of a commercial sod load during 20 hours of storage on the load for Merion Kentucky bluegrass sod harvested on July 10, 1968

Hours on Load	Mean	Distance from Bottom (in)					
		5	10	15	20	25	30
0	67.2	67.5	66.5	68.0	67.5	67.0	67.0
6	69.3	68.5	69.0	69.0	69.5	70.0	70.0
12	70.4	71.0	71.0	70.0	70.0	70.0	70.5
20	73.0	73.0	73.0	73.0	73.0	73.0	73.0

Second Commercial Sod Load

The Merion Kentucky bluegrass sod used in this study was cut between 7 and 8 a.m. and stacked on pallets between 8 and 10 a.m. on May 26, 1969. The effectiveness of ventilation tubes in reducing the rate of temperature increase in the sod stack was investigated. Also, the temperature in relation to distance from the bottom of the palleted sod was measured.

The pallets were 4 by 5 feet in size. One hundred yards of sod were stacked on each pallet. The sod was stacked in alternating layers of flat and rolled pieces of sod. Alternating layers were positioned at right angles to each other. Ventilation tubes were placed on top of the first layer of rolled sod on 2 pallets as the sod was being stacked. The ventilation tubes and elbows were as described for the first sod load, except for being cut to 4 feet in length. Thermocouples and gas sampling tubes were placed in the center of the sod stack at 6, 12, 24, and 36 inches from the bottom of the stack. The thermocouples and gas sampling tube placed at 6 inches from the bottom were 2 sod layers (about 1.6 in) below the ventilation tube. The thermocouple and gas sampling tube placed at 12 inches were one sod layer above the ventilation tube. Two more pallets had thermocouples and gas sampling tubes, but not ventilation tubes, inserted at the same heights. Temperature was measured with a Leeds and Northrup portable potentiometer. The procedure for collection and analysis of gas samples was as described in the Development of Methods section. The temperature and atmosphere within the sod stack were measured at 10 a.m. on May 26 and 6 a.m. on May 27th. The sod load was transported to Cleveland during the late afternoon of May 26, 1969.

Results and discussion.--The main effect means for temperature, percent carbon dioxide and oxygen, and ppm ethylene in relation to distance from the bottom of the sod on pallets with ventilation tubes are presented in Table 58. The ventilation tubes significantly reduced the rate of temperature increase for a distance of 2 inches. Gas levels were not affected by the ventilation tubes. The carbon dioxide and oxygen levels did not change as fast in the palleted sod as in the sod heating boxes. The ethylene level was higher initially than after 20 hours. These facts

Table 58. Temperature and gas levels in relation to distance from the bottom of the sod on pallets with ventilation tubes after 20 hours of storage for Merion Kentucky bluegrass sod harvested on May 26, 1969

Measurement	Initial Mean (0 hr)	Mean (20 hr)	Position of Thermocouples and Gas Sampling Tubes (inches from bottom of sod)			
			6	12	24	36
Temp. (^{o}F)	54	60	57	56	64	64*
% CO_2	2.2	2.0	2.4	2.0	2.2	1.6
% O_2	7.2	18.4	17.8	18.2	18.8	18.7
ppm C_2H_4	2.8	1.3	1.5	1.0	1.4	1.2

*Differences between main effect means are significant at the .05 level of probability.

indicate that the gas diffusion rate was greater in the sod load than in the sod heating boxes. The 10^{o} F temperature increase during 20 hours of storage was similar to rates of temperature increase in sod heating boxes.

The main effect means for temperature and gas levels in relation to distance from the bottom of the sod on pallets without ventilation tubes are presented in Table 59. The initial temperature of this sod was higher because it was stacked about an hour later. The rate of temperature increase was slow. Carbon dioxide accumulated significantly faster near the bottom of the sod stack. Oxygen percentages were lower and temperatures were higher near the bottom of the sod stack, but not significantly different from values

Table 59. Temperature and gas levels in relation to distance from the bottom of the sod on pallets after 20 hours of storage for Merion Kentucky bluegrass sod harvested on May 26, 1969

Measurement	Initial Mean (0 hr)	Mean (20 hr)	Position of Thermocouples and Gas Sampling Tubes (inches from bottom of sod)			
			6	12	24	104
Temp. (^oF)	61	64	65	65	62	64
% CO_2	2.6	3.3	4.3	4.8	2.0	2.0**
% O_2	16.1	16.2	15.4	14.7	17.3	17.6
ppm C_2H_4	2.7	1.4	1.5	1.6	1.0	1.6

**Differences between main effect means are significant at the .01 level of probability.

found at the 24 and 36 inch positions of measurement. The carbon dioxide and oxygen levels did not change as rapidly in the palleted sod as in the sod heating boxes. The ethylene level was lower after 20 hours of storage than initially. The gas diffusion rate was greater in the sod load than in the sod heating boxes. The sod survived shipment without injury.

Third Commercial Sod Load

The Merion Kentucky bluegrass sod used in this study was cut and rolled between 11 and 12 a.m. and stacked on pallets between 3 and 4 p.m. on June 9, 1969. The temperature increase in relation to distance from the bottom of 4 pallets was measured. Thermocouples and gas sampling tubes were placed at 6, 12, and 24 inches from the bottom of the sod stacks. Temperature and atmosphere within the sod stack were measured at 4:30 p.m. June 9th and at 4:30 a.m. on June 10, 1969. The sod load was transported to Cleveland during that night.

Results and discussion.--The main effect means for temperature, percent carbon dioxide and oxygen, and ppm ethylene are presented in Table 60. The temperature and gas measurements did not differ in relation to the distance from the bottom of the pallets. The temperature increased 9^{o} F during 12 hours on the load. This rate of temperature increase was similar to that occurring in sod heating boxes. The rate of change in carbon dioxide and oxygen levels was faster in this load than in previous sod loads, but not as rapid as in the sod heating boxes. Ethylene levels were very low. The sod survived shipment without any injury.

Table 60. Temperature and gas levels in relation to distance from the bottom of the sod on pallets after 12 hours of storage for sod harvested on June 9, 1969

Measurement	Initial Mean (0 hr)	Mean (12 hr)	Position of Thermocouples and Gas Sampling Tubes (inches from bottom of sod)		
			6	12	24
Temp. (^{o}F)	77	86	86	87	86
% CO_2	2.9	10.1	10.6	10.4	9.3
% O_2	17.1	7.8	6.7	7.9	8.8
ppm C_2H_4	0.30	0.20	0.18	0.25	0.18

Comparison of Sod Load and Sod Heating Box Conditions

The rate of temperature increase was nearly identical for the third sod load and Experiment I (May 16, 1969). The initial sod temperature was 77° F compared to 86° F after 12 hours in the third sod load. The initial temperature was 73.5° F compared to 82.1° F after 12 hours in Experiment I. The initial sod temperature was 69° F compared to 77° F after 12 hours in Experiment IV (June 4, 1969). The initial sod temperatures and the rates of temperature increase varied among sod heating box experiments. The initial sod temperature and rate of temperature increase was lower in the first 2 commercial sod loads than for sod heating box experiments. Nevertheless, the data clearly shows that the sod heating boxes simulated commercial sod load conditions quite well in terms of heat exchange.

The carbon dioxide level was 10% after 12 hours in the third sod load compared to 16% after 12 hours in Experiment IV. The rate of increase of carbon dioxide level was slower in the second sod load. The oxygen levels were higher after 12 hours in the sod loads than in sod heating box experiments. Therefore, the sod heating boxes were tighter in terms of gas exchange than the palleted sod.

MEASUREMENT OF RESPIRATION RATES

Respiration rates of sod were measured on the Automatic Photosynthetic and Respiration Integrating Laboratory (APRIL) in the Department of Horticulture (Dilley, 1969). Three 6 inch diameter sod pieces were spaced in a triangular pattern in the bottom of bucket chambers similar to those described for the controlled atmosphere experiments. The chambers were placed in a constant temperature room at 27° C. The respirometer chambers were connected through a system of tubing and valves to a Beckman IR-115 analyzer. A continuous flow of air at 300 ml/hr was maintained in the system. The ppm carbon dioxide in the airstream from each chamber of the series was measured at 7 minute intervals in a 12 hour cycle. The respiration rate was calculated with the following equation:

$$\text{ml } CO_2/\text{kg/hr} = \frac{(\text{Flow in ml/hr})\,(273)\,(P)}{(\text{Weight in kg})\,(T)\,(760)}\,(\%\ CO_2 \text{ in sample} - \%\ CO_2 \text{ in air blanks})$$

The respiration rate in terms of oxygen use was not obtained because the Beckman G-2 Oxygen Analyzer was not working properly.

Respiration Rate of the Sod Used in Experiment IV

The respiration rate was measured on sod that had received the following cultural treatments: (a) 2 versus 0.75 inch cutting heights, (b) 0 versus 215 lb/A of nitrogen, and (c) 0 versus 0.0550 lb/A of N^6benzyladenine. The low cutting and nitrogen fertilization was done 5 days before harvest. N^6benzyladenine was applied just before harvest. The sod cut at 2 inches had many seedheads present. The sod pieces were harvested in the forenoon and kept covered in the shade until they were placed in respirometer chambers in the late afternoon of June 4, 1969. Respiration measurements were obtained at 10 p.m. June 4, and at 10 a.m. and 10 p.m. June 5.

Results and discussion.--The respiration rates in relation to cutting height, nitrogen rate, and N^6benzyladenine treatments are presented in Table 61. The mean rate of respiration decreased slowly over time, possibly as a result of slow depletion of available carbohydrates. The low cutting resulted in a decreased respiration rate. This was expected since the relative proportion of living tissue to weight of sod pieces was lower after low cutting. The reduced respiration rate with low cutting corresponds to the decreased temperature, carbon dioxide, and injury levels found in the sod heating box experiment. The high nitrogen rate resulted in higher numerical values for respiration rate, but the differences were not statistically significant. N^6benzyladenine did not affect respiration rate significantly.

Table 61. Respiration rate in relation to cutting height, nitrogen rate, and N6benzyladenine treatments of Merion Kentucky bluegrass sod harvested on June 4, 1969 (Experiment IV)

Date and Time of Measurements	Mean	Respiration Rate (ml CO_2/kg/hr)					
		Cutting Ht. (in)		Nitrogen (in)		N^6BA (lb/A)	
		2	0.75	0	215	0	.055
June 4, 10 p.m.	108	121	97†	104	113	113	104
June 5, 10 a.m.	103	114	93†	97	109	106	100
June 5, 10 p.m.	98	106	89	92	103	101	96

†Differences between main effect means are significant at the .10 level of probability at designated times of measurement.

Components of Respiration

The respiration rate of complete sod pieces and organic soil was measured for Merion Kentucky bluegrass sod and soil harvested on June 7 and 14, 1969. The sod was from Green Acres Sod Farm; the same sod source as for the first seven 1969 sod heating box experiments. Only a few seedheads were present in the sod during these experiments. The cutting height of the sod was 2 inches. The sod was cut at a 0.7 inch depth and then into 6 inch diameter pieces. Three of the sod pieces were placed in each chamber. The organic soil was obtained by scraping sod pieces over screen having 0.25 sq inch openings. These sod pieces were discarded. A 0.6 inch layer of this soil was spread over the bottom of the respirometer chambers. Four replications were used.

Air pump failure in APRIL prevented measurement of respiration of sod and soil collected on June 7 until June 9 at 10 a.m. and 10 p.m. The chambers were left open in the 27^{o} C room until the air pump was replaced.

The respiration rate of the sod and soil collected on June 14 was measured at 10 a.m. and 10 p.m. on June 15.

Results and discussion.--The respiration rates for sod and organic soil harvested on June 7 and 14 are presented in Table 62. The respiration rate of the sod was about 8 times greater than that of the organic soil in which sod had been growing. One of the implications of this data

Table 62. Respiration rates for Merion Kentucky bluegrass sod cut at 2 inches and organic soil harvested on June 7 and 14, 1969

Date and Time of Measurements	Respiration Rate (ml CO_2/kg/hr)	
	Sod	Soil
June 9, 10 a.m.	80	13***
June 9, 10 p.m.	87	12***
June 15, 10 a.m.	69	4***
June 15, 10 p.m.	62	10***
Overall Mean	75	10

***Differences between main effect means are significant at the .001 level of probability for designated times of development.

is that lower cutting of the turf just before harvest will be more effective in reducing respiration rate (and therefore temperature increase) in the load than thinner cutting of the sod. This is due to the fact that the respiration rate of the turfgrass plants is about 6.5 times that of the soil microorganisms.

SUMMARY AND DISCUSSION

Commercial sod is composed of living turfgrass plants whose roots and rhizomes are tightly intertwined in a thin layer of soil. The plants continue to respire during shipment. Respiration uses oxygen and reserve carbohydrates and releases carbon dioxide and heat. If these changes occur rapidly in the load injury or death of the turfgrass plants may occur before the sod is unloaded and transplanted. Usually sod will survive in the load 24 hours or sometimes longer without injury, but incidents of sod damage in 12 hours or less have occurred.

Dissipation of heat and diffusion of oxygen and carbon dioxide are severely limited within the confined space of the sod load. Information on the changes in temperature, gas levels, and carbohydrate reserves during storage under simulated shipping conditions was obtained from the sod heating box experiments. Sod response to carbon dioxide, oxygen, and ethylene levels in controlled atmospheres was investigated. The major implications of these results will be discussed in the following sections.

Effects of Cultural Treatments on Sod During Storage

The effects of height of cut, nitrogen fertilization, respiration inhibitors, and time of day of harvest were investigated in a series of sod heating box experiments. The sod heating boxes simulated commercial sod load conditions well in terms of temperature, but were more restrictive of gas diffusion. The following sections summarize the results of the experiments.

Height of cut.--The 2 inches versus 0.75 inch height of cut treatment was included in 7 of the 1969 sod heating box experiments. The 0.75 inch height of cut resulted in lower temperatures. The 0.75 inch height of cut reduced carbon dioxide levels by 1 to 4% in most experiments. The oxygen levels were higher during the early hours of storage where the sod was cut at 0.75 inch. The ppm ethylene was lower for sod cut at 0.75 inches. The 0.75 inch height of cut treatment never increased temperature, carbon dioxide or ethylene levels. Low cutting greatly reduced the amount of respiring leaf tissue and this provides a satisfactory explanation for these results.

The mean respiration rate was about 115 ml CO_2/kg/hr for sod cut at a 2 inch height in Experiment IV where abundant seedheads were present. The mean respiration rate was 93 ml CO_2/kg/hr where most of the seedheads were removed by the 0.75 inch cutting treatment for sod used in Experiment IV. The mean respiration rate was 75 ml CO_2/kg/hr for sod cut at a 2 inch height (no seedheads present) and used in the components of respiration studies. The respiration rate of sod is greater when seedheads are present and reduced by lowering the height of cut.

The 0.75 inch height of cut treatments were initiated 10, 5, and 1 day before harvest and 10 and 5 days before harvest and on the day of harvest for Experiment III and VII, respectively. The respiration rate and temperature was slightly lower for sod cut at 0.75 inch 10 days before harvest, but slightly less injury and more leaf cover and root production occurred for sod cut at 0.75 inch on the day of harvest or 1 day before harvest.

The effects of the 0.75 height of cut on percent leaf kill and cover and root production appear to be more closely related to temperature than to height of cut *per se*. In general, when the mean temperatures for an experiment were above 92^{o} F the 0.75 inch height of cut resulted in lower temperatures which reduced injury and increased root production.

Nitrogen fertilization.--The effects of nitrogen fertilization on sod heating and damage were studied in 6 of the sod heating box experiments. The effects of applying 215 lb/A of nitrogen a few days before harvest were investigated in five experiments. The application of 215 lb/A of nitrogen is 2 to 5 times more than a commercial sod producer should apply at one time. The "zero" nitrogen rate was the amount applied in the normal sod production fertilization program; that is, 100 to 150 lb/A/year depending on the sod source.

Significantly higher temperatures resulted from the 215 lb/A nitrogen application for the June 4, 1969 experiment. Abundant seedheads were present. No differences in temperature in relation to nitrogen level were found for other experiments which compared the "zero" and 215 lb/A nitrogen level.

Generally, the carbon dioxide level was increased and the oxygen was decreased somewhat during the early hours of storage as a result of the 215 lb/A nitrogen treatment.

The 215 lb/A nitrogen treatment resulted in higher levels of ethylene in all 5 experiments where "zero" versus 215 lb/A nitrogen levels were compared.

The percent leaf kill was greater and the percent leaf cover and root production were lower where the 215 lb/A nitrogen was applied. This general conclusion is valid although the results for individual experiments after given times of storage were neither perfectly consistent nor always significant.

Temperature, carbon dioxide, and leaf kill increased progressively with nitrogen rate while leaf cover and root production decreased progressively in relation to nitrogen rate in Experiment X. Sod which had been produced with 0, 90, 180, 360, and 340 lb/A of nitrogen was compared. The commercial sod (340 lb/A of N) had the best appearance and seemed to be growing the fastest. The other four sods were produced on a low fertility organic soil. The sod grown with 0 or 90 lb/A of nitrogen had better root production after storage than the other sods.

Respiration inhibitors.--A respiration inhibitor which could be sprayed onto sod shortly before harvest to reduce injury during shipment would be an ideal solution to the sod heating problem. N^6benzyladenine, a respiration inhibitor capable of prolonging storage life of green leafy vegetables (Dedolph, Wittwer, Tuli, and Gilbert, 1962) showed some promise in preliminary investigations. Alar*85 (succinic acid* 2,2-dimethyl hydrazide, 85% by weight) and Cycocel (CCC) (2-(chloroethyl)trimethylammonium chloride) did not show any effect in preliminary trials.

N^6benzyladenine, applied at a rate of 0.055 lb/A in 65 gal of water per acre, was included in 6 of the 1969 experiments. N^6benzyladenine at 0.0055, 0.0275, and 0.0550 lb/A rates was sprayed onto sod just prior to harvest for Experiment I. The 0.055 lb/A rate resulted in a decrease in temperature after 96 hours of storage. The 0.055 lb/A rate of N^6 benzyladenine in combination with a 0.75 inch height of cut also reduced temperature significantly after 48, 72, and 96 hours of storage. Because of these results the 0.055 lb/A of N^6benzyladenine was used in subsequent experiments.

Generally, N^6benzyladenine did not affect the results of the experiments. Thirteen scattered instances of significant differences between main effect means for 0 and 0.055 lb/A of N^6benzyladenine occurred as follows: temperature and carbon dioxide were decreased once each, oxygen was increased twice, ethylene was increased once and decreased once, percent leaf cover was decreased once, and root production was decreased twice. The N^6benzyladenine was applied just prior to harvest and 5 and 10 days before harvest in Experiment VI and none of the main effect means for N^6benzyladenine treatments were different. In several instances N^6benzyladenine interacted significantly with height of cut or nitrogen level to affect temperature, oxygen or ethylene levels, but these trends were not consistent either. One must conclude that N^6benzyladenine was not shown to be of practical value for reducing sod injury during shipment.

Time of harvest.--The effects of 9 a.m. versus 2 p.m. harvest were investigated in Experiment IX. The initial temperatures were 73.5 and 83.1° F. The sod harvested in the morning was cooler during the entire 72 hours of storage, but its rate of heating was faster than for sod harvested in the afternoon. Percent leaf kill and cover and root production were not affected by the temperature differences probably because of the unusually rapid rate of injury (61% leaf kill after 24 hours). Nevertheless, early morning harvest should normally prolong the safe storage of sod.

Physiological Mechanisms Involved in Sod Injury

Physiological processes that might have caused or contributed to sod injury during storage under simulated shipping conditions include suffocation, ethylene toxicity, carbohydrate starvation, and high temperature injury. The following sections discuss these possibilities.

Suffocation.--No evidence for suffocation under the conditions in the sod heating boxes was found. The carbon dioxide in the sod heating boxes increased rapidly to 13 to 19%. The oxygen decreased concurrently to 2 to 5%. These changes in carbon dioxide and oxygen levels occurred within 24 hours. The carbon dioxide and oxygen levels remained relatively constant for longer periods of storage. The controlled atmosphere studies showed that root production was highest after 120 hours of storage for the 18% CO_2 and 2% O_2 treatments. The 4 and 8% O_2 levels resulted in better root production for shorter periods of storage. The 16% O_2 level reduced root production. The 0, 9, and 27% CO_2 levels resulted in less root production than for 18% CO_2. Leaf injury was not observed after CA treatment with any gas mixture that contained 2% or more of oxygen. Percent leaf kill was not affected by the carbon dioxide and oxygen levels that occurred in the sod heating boxes. Root production may have been decreased somewhat as a result of low oxygen levels in some experiments (especially likely in Experiment V). Actually, the carbon dioxide and oxygen levels that occurred in the sod heating boxes were near optimum for the longest survival of sod in storage.

However, the carbon dioxide levels were lower and the oxygen levels remained higher under commercial sod load conditions than in the sod heating boxes. The higher oxygen level may be beneficial for up to normal length of storage time, but it will probably result in greater injury during adverse conditions or longer than usual times of storage on commercial sod loads.

Ethylene toxicity.--Ethylene toxicity undoubtedly contributed to sod injury in some of the sod heating box experiments, but it would rarely cause injury in commercial sod loads.

The controlled atmosphere studies showed that 0 and 2 ppm of ethylene did not reduce root production but that 4 and 8 ppm did. A definite threshold level for ethylene injury exists between 2 and 4 ppm of ethylene.

High ethylene levels in the sod heating box experiments were generally associated with the high (215 lb/A) nitrogen treatments. The ethylene levels exceeded 3 ppm (highest was 5.35 ppm) for the 215 lb/A nitrogen treatments after 12, 24, and 48 hours of storage in Experiment V. Root production was quite low for the high nitrogen treatments after 48 hours and zero after 72 hours in Experiment V. Ethylene production above 2 ppm was associated with high nitrogen treatments in Experiments IV and IX. Ethylene production may result from a minor anaerobic respiration pathway accentuated by high concentrations of ammonium ion in the grass plant. Apparently more ethylene was produced when the oxygen level was slightly below 2% as occurred in Experiment V. Ethylene production was independent of temperature. The highest ethylene levels were found after 24 or 48 hours of storage in the sod heating box experiments.

Factors other than excessive nitrogen fertilization can result in high ethylene levels. A 3.22 ppm ethylene level was associated with the 2 inch height of cut after 24 hours of storage during Experiment VIII. A mean of 2.66 ppm of ethylene occurred with the "zero" nitrogen rate after 24 hours of storage during Experiment III.

The ethylene levels remained below 2 ppm in the other experiments. The nitrogen level was equal to or below that commonly applied in producing commercial sod in most of these experiments. Therefore, it is concluded that ethylene toxicity would not generally be a cause of sod injury in commercial sod loads.

Carbohydrate starvation.--The decrease in percent total available carbohydrates (TAC) correlated closely with the increases in percent leaf kill during 13 days of storage in the sod heating boxes during Experiment XI. The TAC decreased from 28.6 to 12.8% while the leaf kill increased from 0 to 100%. The fructosan content of the stems was 5.3, 5.3, and 6.9% after 9, 11, and 13 days of storage, respectively. Sullivan and Sprague (1949) clipped perennial ryegrass plants to 1.5 inches and then grew the plants at 90/80° F and 525 ft-c in growth chambers for 40 days. The fructosan content in the stubble decreased from 28 to 7% during the first 21 days. Even though the plants were stunted and spindly, they survived for 19 more days with the 7% level of fructosan.

The percent TAC in relation to percent leaf kill and root production for sod stored in controlled atmospheres at 104 and 83° F was measured. This data did not show a correlation between percent TAC and percent leaf kill or temperature.

The data show that TAC was depleted during sod storage, but TAC was not exhausted or depleted to a consistent low level before death of the sod occurred. Carbohydrate starvation was not a direct cause of sod death. Whether or not carbohydrate depletion contributed to sod injury can not be determined from the limited data gathered.

High temperature injury.--The growth and development of turfgrasses is usually confined to a 40 to 105° F temperature range (Beard, in press). The optimum temperature range for sustained root growth for Kentucky bluegrass is 50 to 65° F (Brown, 1943). Kentucky bluegrass root and rhizome growth is restricted at soil temperature above 90° F (Beard, in press). Mitchell (1956) found that growth of cool season grasses ceased above 95° F. Fischer (1967) found that temperatures above 105° F killed *Poa annua*.

Soil temperatures are more important than air temperatures in plant survival (Carroll, 1943). The lethal temperature varies with the time of exposure; the lower the temperature the longer the time required to produce tissue kill (Fischer, 1967).

A summarization of mean temperatures, percent leaf kill, and root production for the 1969 sod heating box experiments is presented in Table 63. In general, the higher the temperature the greater the percent of leaf kill. Root production was greater and temperatures were lower in Experiments I and II. The root production was lower and less consistent in the later experiments. High nitrogen treatments seemed to result in somewhat more injury for a given temperature level. Pellett and Roberts (1963) found that high nitrogen reduced the ability of Kentucky bluegrass to resist high temperature injury. The presence of seedheads increased the injury in relation to temperature in Experiment IV. The high initial soil temperature resulted in more rapid increases in leaf kill in Experiment VIII; probably because of the longer exposure to high temperature. The controlled atmosphere temperature study showed very clearly that the 104° F temperature resulted in more leaf kill and less root production than for 83° F. One must conclude that sod injury is closely related to temperature level. The higher the temperature the more sod injury occurred.

Table 63. Summarization of mean temperatures, percent leaf kill, and root production for the 1969 sod heating box experiments

Experiments and Treatments	Hours of Storage	Mean Temp. (°F)	Mean % Leaf Kill	Mean Root Production (mg/pot)
	0	74		
I	24	91	0	46
2 vs 0.75 inch cut	48	91	0	102
N^6BA	72	88	0	82
	96	86	0	40
II	6	71		
2 vs 0.75 inch cut	24	82	0	90
0 vs 215 lb N	48	86	0	82
N^6BA	72	86	0	92
	96	86	6	49
	0	85		
III	24	94	0	49
2 vs 0.75 inch cut	48	95	0	47
10, 5, 1 days	72	95	9	35
N^6BA	96	93	45	18
	120	87	91	20
IV	0	69		
2 vs 0.75 inch cut	24	81	0	34
0 vs 215 lb N	48	87	6	31
N^6BA	72	88	41	32
(seedheads)	96	89	86	6
V	0	74		
0 vs 215 lb N	24	88	9	60
4, 8, 18 days	48	93	8	9
N^6BA	72	90	90	14
	00	68		
VI	24	82	0	43
2 vs 0.75 inch cut	48	88	1	93
0, 5, 10 days	72	91	3	110
N^6BA	96	90	30	79
	0	69		
VII	24	78		
2, 0.75, 0.50 inch cut	48	88	10	40
0.75--0, 5, 10 days	72	93	23	75
	96	94	51	38
VIII	0	87		
2 vs 0.75 inch cut	24	95	29	8
0 vs 215 lb N	48	95	84	20
N^6BA	72	95	94	8
	0	78		
IX	24	90	61	135
9 a.m. to 2 p.m.	48	94	98	8
0, 130, 215 lb N	72	96	100	0
	0	82		
X	24	90	2	64
0, 90, 180,	48	93	7	47
360, 340 lb N	72	93	16	54
during production	96	95	48	34
	120	94	69	33

CONCLUSIONS

The following conclusions may be drawn from these experiments.

1. Sod injury increased progressively in relation to increased temperature levels occurring during storage of Merion Kentucky bluegrass sod under simulated shipping conditions.
2. Sod cut at a 0.75 inch height within a few days before harvest survived storage longer than sod cut at 2 inches.
3. Sod injury during storage increased progressively with increasing rates of nitrogen fertilization.
4. Inhibition of respiration from oxygen starvation or from high carbon dioxide levels was not a cause of sod injury.
5. Early morning harvest increases the length of time that sod may be stored in the load.
6. N^6benzyladenine, a respiration inhibitor, did not affect carbon dioxide and oxygen levels, temperature, or injury of sod during storage.
7. The amount of ethylene released in commercial sod loads during shipment is too small to cause injury of sod.

8. The rate of respiration was higher and the amount of sod injury was greater relative to temperature when seedheads were present.
9. Root production decreased as temperature and nitrogen levels increased.

BIBLIOGRAPHY

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Beard, J. B. In press. The principle of turfgrass culture. Prentice Hall, Inc., New Jersey.

Beard, J. B., and R. C. Hoglund. 1966. The value and acreage of turf in Michigan. Compiled by the Departments of Crop Science and Agricultural Economics at Michigan State University. (Unpublished) Summary published in Michigan AES Science in Action No. 2--Turf and Sod, 1969.

Bernfeld, Peter. 1951. Enzymes of starch degradation and synthesis. Adv. Enzymology 12: 379-428.

Blom, L., and L. Edelhausen. 1955. Direct titration of carbon dioxide. Analytica Chimica Acta 13: 120-128.

Brown, E. M. 1943. Seasonal variations in the growth and chemical composition of Kentucky bluegrass. Missouri Agr. Exp. Sta. Bull. 360, pp. 1-56.

Carroll, J. C. 1943. Effects of drought, temperature and nitrogen on turf grasses. Plant Physiol. 8: 19-36.

Dedolph, R. R., S. H. Wittwer, V. Tuli, and D. Gilbert. 1962. Effect of N^6benzylaminopurine on respiration and storage behavior of broccoli (*Brassica oleracea* var. *italica*). Plant Physiol. 37: 509-512.

Dilley, David R., D. H. Dewey, and R. R. Dedolph. 1969. Automated system for determining respiratory gas exchange of plant materials. J. Am. Soc. for Hort. Sci. 94: 138-141.

Fischer, James Alan. 1967. An evaluation of high temperature effects on annual bluegrass (*Poa annua* L.). M.S. thesis. Michigan State University, pp. 1-42.

Mitchell, K. J. 1956. Growth of pasture species under controlled environment. I. Growth at various levels of constant temperature. New Zealand J. Sci. Technol. A. 38: 203-216.

Okajima, Hideo, and Dale Smith. 1964. Available carbohydrate fractions in the stem bases and seed of timothy, smooth bromegrass and several northern grasses. Crop Sci. 4: 317-320.

Pellett, H. M., and E. C. Roberts. 1963. Effects of mineral nutrition on high temperature induced growth retardation of Kentucky bluegrass. Agron. J. 55: 473-476.

Satari, Achmad M. 1967. Effects of various rates and combinations of nitrogen, phosphorus, potassium and cutting heights on the development of rhizome, root, total available carbohydrate and foliage composition of _Poa pratensis_ L. Merion grown on Houghton muck. Ph.D. thesis, Michigan State University, pp. 1-127.

Smith, Dale, G. M. Paulsen, and C. A. Raguse. 1964. Extraction of total available carbohydrates from grass and legume tissue. Plant Physiol. 24: 706-719.

Sullivan, J. T., and V. G. Sprague. 1949. The effect of temperature on the growth and chemical composition of the stubble and roots of perennial ryegrass. Plant Physiol. 24: 706-719.

Youngner, V. B., and F. J. Nudge. 1968. Growth and carbohydrate storage of three _Poa pratensis_ L. strains as influenced by temperature. Crop Sci. 8: 455-457.