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Brown Stain Susceptibility of Selected Lettuce Cultivars Under Controlled Atmospheres and Temperatures[1, 2]

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Abstract. Laboratory tests of 11 cultivars of crisphead type lettuce (*Lactuca sativa* L.) indicated all were susceptible to brown stain. Susceptibility varied among cultivars, with 'Great Lakes 66', 'Calmar', 'Valrio', 'Merit' and 'Great Lakes R-200' the most susceptible and 'Greenland', 'Climax' and 'Francisco' the least affected. Storage temperature markedly influenced brown stain incidence: lettuce held at 0°C developed more brown stain than that held at 2.5°C. Development of the disorder required that lettuce be subjected to elevated CO_2 in storage. Brown stain incidence for a given cultivar varied with date of harvest and production area.

The transition of more tightly sealed railcars and trailers for commercial shipment of lettuce, with or without the intentional modification of the atmosphere, has brought with it the problem of increased CO_2 levels in these vehicles. As early as 1931 levels of CO_2 above 7% were reported to be injurious to lettuce held for 7 days at 0° or 4°C (Thornton, 1931). Increased CO_2 has been reported to cause various physiological disorders of lettuce (Rappaport, 1957; Watada et al., 1964). The symptoms reported included variable sized reddish-brown pitted spots along the midrib. In 1970, the name "brown stain" was given to physiological disorder associated with increased CO_2 in the range of 2½ to 10% (Stewart, et al., 1970). Precise descriptions and illustrations of brown stain symptoms have been given (Stewart et al., 1970; Lipton, et al., 1972). Carbon monoxide (CO) in the presence of elevated CO_2 increased lettuce susceptibility to brown stain (Stewart and Uota, 1972).

The market quality of head lettuce has been evaluated after storage in low O_2 atmospheres which retard russet spotting (Watada et al., 1964; Lipton, 1967; Parsons et al., 1964; Singh et al., 1972) and butt discoloration (Parsons et al., 1964 Singh et al., 1972) but increase brown stain (Stewart and Uota 1971). In contrast, 2.5% O_2 and 2.5% CO_2 at 2.4°C was reported to be the best combination of gases for lettuce storage (Singh et al., 1972).

Extensive research has shown that lettuce deteriorates more rapidly at high temp than at low temp (Pratt et al., 1954, Lipton, 1967; Parsons and Wright 1956, Singh et al., 1972; Stewart and Harvey, 1966 and 1967; and Watada et al., 1964), but so far the influence of temp on susceptibility of lettuce to brown stain has been unreported.

Studies were initiated in 1970 to evaluate various preharvest and postharvest variables on brown stain development. Early in this study differences among cultivars were noted and we undertook comparative studies. Since submission of this manuscript, information has been presented on relative cultivar susceptibility to brown stain (Stewart and Matoba, 1972). Our study considers the susceptibility of selected lettuce cultivars to brown stain as affected by temp, composition of the atmosphere (CA), and season of harvest.

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Materials and Methods

Experimental material. Eleven cultivars of lettuce (*Lactuca sativa* L.), (Table 1) were harvested in 1971 from El Centro, Kettleman City, and Westmorland, California, and from Parker, Arizona. Harvest dates were between January 22 and May 1.

Table 1. The effect of brown stain inducing treatments on various lettuce cultivars.

Treatment[y] O_2 (%)	CO_2 (%)	Temp (°C)	Cultivar[z]	Harvest date and growing area: 1/22/71 El Centro Calif.	2/11/71 El Centro Calif.	3/4/71 Westmorland Calif.	3/25/71 Parker Ariz.	4/15/71 Kettleman Calif.	5/15/71 Kettleman Calif.	Brown Stain Index across experiments[w]
				Brown Stain Index[x]						
2	5 (T_3)	2.5	Calmar	32			16	52	14	29
			Merit	14	1					8
			Valrio	65				62		64
			Golden State D	19			4	8		10
			Forty-Niner	15			4		11	10
			Great Lakes 66	79						79
			Great Lakes R-200	39			10	4	3	14
			Greenland		0	19				10
			Vanguard		0	1	1	0	7	2
			Climax		0	0	1	1	6	2
			Francisco			0				0
2	10 (T_1)	2.5	Calmar	143			77	66	109	99
			Merit	72	68					70
			Valrio	120				65		93
			Golden State D	76			43	35		51
			Forty-Niner	96			39		15	50
			Great Lakes 66	104						104
			Great Lakes R-200	102			64	43	90	75
			Greenland		1	23				12
			Vanguard		35	3	73	29	105	49
			Climax		48	19	20	24	49	32
			Francisco		37					37
2	5 (T_2)	0	Calmar	116			48	48	52	66
			Merit	83	46					65
			Valrio	105				40		73
			Golden State D	86			9	45		47
			Forty-Niner	91			13		25	43
			Great Lakes 66	98						98
			Great Lakes R-200	86			27	8	69	48
			Greenland		7	6				7
			Vanguard		12	1	30	5	42	18
			Climax		51	4	14	47	28	29
			Francisco			14				14
LSD		0.05		27	24	20	30	26	25	

[z]Eight heads of lettuce were used for each treatment-cultivar combination.

[y]No brown stain was observed in control treatments (air and 2% O_2, 0% CO_2 at 2.5°C).

[x]The brown stain index is based on the discoloration, size and number of lesions. Values shown represent the mean index of 8 replicates. Observations were made after a 4-day period under air flow at 10°C following CO_2 treatments at 0° or 2.5°C. See text for details.

[w]Values shown represent the mean index of all replicates for a given treatment-cultivar combination. Error mean square from unequal cell frequency 1-way ANOVA if 1089; t statistic (0.05) = 1.960.

The lettuce was vacuum-cooled and then transported to Davis, California in refrigerated or insulated vehicles. On arrival, the lettuce was inspected for brown stain and other defects. Uniform quality heads were used in all experiments.

Treatment design. Five standardized treatments were used to determine the relative susceptibility of the culitvars to brown stain. The treatments are identified in the text as follows:

Designation	Treatment: O_2 %	CO_2 %	Temp (°C)
T_1	2	10	2.5
T_2	2	5	0
T_3	2	5	2.5
T_4	2	0	2.5
T_5	21	0	2.5

Prior to storage of the lettuce, wrapper leaves were removed and 8 randomly selected heads of a given cultivar were placed in a container (one container per treatment). The containers were held at the desired temp (±0.5°C) and were ventilated with a humidified gas stream of known composition and a flow rate that resulted in an increase of about 0.25% CO_2 in the effluent. The gases were monitored by means of a gas chromatograph, infrared analyzer, or Beckman G-2 O_2 analyzer.

At the end of a 10-day treatment period, the lettuce was inspected for general quality and externally visible brown stain. The heads were then placed back in the containers, which were closed and transferred to 10°C and air flow for 4 days after which time the heads were inspected for external and internal brown stain. Brown stain was evaluated according to the scoring system:

Score (per head)	Lesions: Estimated size (inches)	Number	Discoloration
0	None	None	z
1	$<$ 1/4	1-2	None
2	1/4–1/2	3-6	Slight
3	$>$ 1/2–3/4	7-10	Moderate
4	$>$ 3/4– 1	10-15	Severe
5	$>$ 1–1 1/2	$>$ 15	Extreme
6	$>$ 1 1/2	z	z

[z]Not applicable

An index was derived from the product of discoloration score, number score, and size score [discoloration x number x size]. The relative effect of brown stain incidence on quality is shown below.

Brown stain index	*Visual quality*
2-30	Slight effect
30-60	Moderate effect
60-90	Severe effect
>90	Unsalable

Statistical analysis. Each experiment was analyzed by 1-way analysis of variance (ANOVA). If significant differences at the 5% level were indicated by the F test, then the Least Significant Difference Test (LSD) was used to determine what treatment-cultivar combinations were significantly different. Differences among treatment-cultivar combinations across all experiments were determined by 1-way ANOVA for unequal cell frequencies.

Results and Conclusions

The severity of brown stain resulting from a given CO_2 treatment varied greatly among tests with different harvest dates. This variability in brown stain development has been characteristic of our general results and has been mentioned by others (Stewart and Uota, 1972). Despite this variability, differences in brown stain expression are attributable to cultivar, to CO_2 concn, and to temp of treatment.

Effect of CO_2. In these tests, brown stain developed only following treatments with added CO_2. Hence, only treatments T_1, T_2, and T_3 were considered in the reduction and presentation of the data. Comparing the 5% and 10% levels of CO_2 at 2.5°C (T_3 vs. T_1), brown stain was greatest for the 10% treatment in every case and the increase was statistically significant in 23 of the 32 possible comparisons (Table 1). If each cultivar is averaged across all tests in which it was included. the marked difference between 5% and 10% CO_2 is seen (Table 1 and Fig. 1). Nine of the 11 cultivars showed nil to slight brown stain at 5% CO_2; in contrast 10 of the 11 cultivars showed moderate, or greater, brown stain following exposure to 10% CO_2.

Effect of temp. The severity of brown stain as a symptom of CO_2 injury is dependent upon the temp during exposure. The temp dependence of injury induction and symptom development will be clarified in a subsequent paper. The tests reported here permit comparison of the effects of 0° vs. 2.5°C for the 5% CO_2 level (T_2 vs. T_3). In 32 possible comparisons, brown stain was greater at 0° in 28 and the difference was significant in 15 (Table 1). When averaged across tests, 10 of the 11 cultivars showed more brown stain following exposure to 0°C. 'Greenland' was the exception and showed only slight brown stain at each temp. Thus the generalized effect of increased brown stain by low temp is established for a wide spectrum of cultivars.

Response of cultivars. Cultivars differed in their

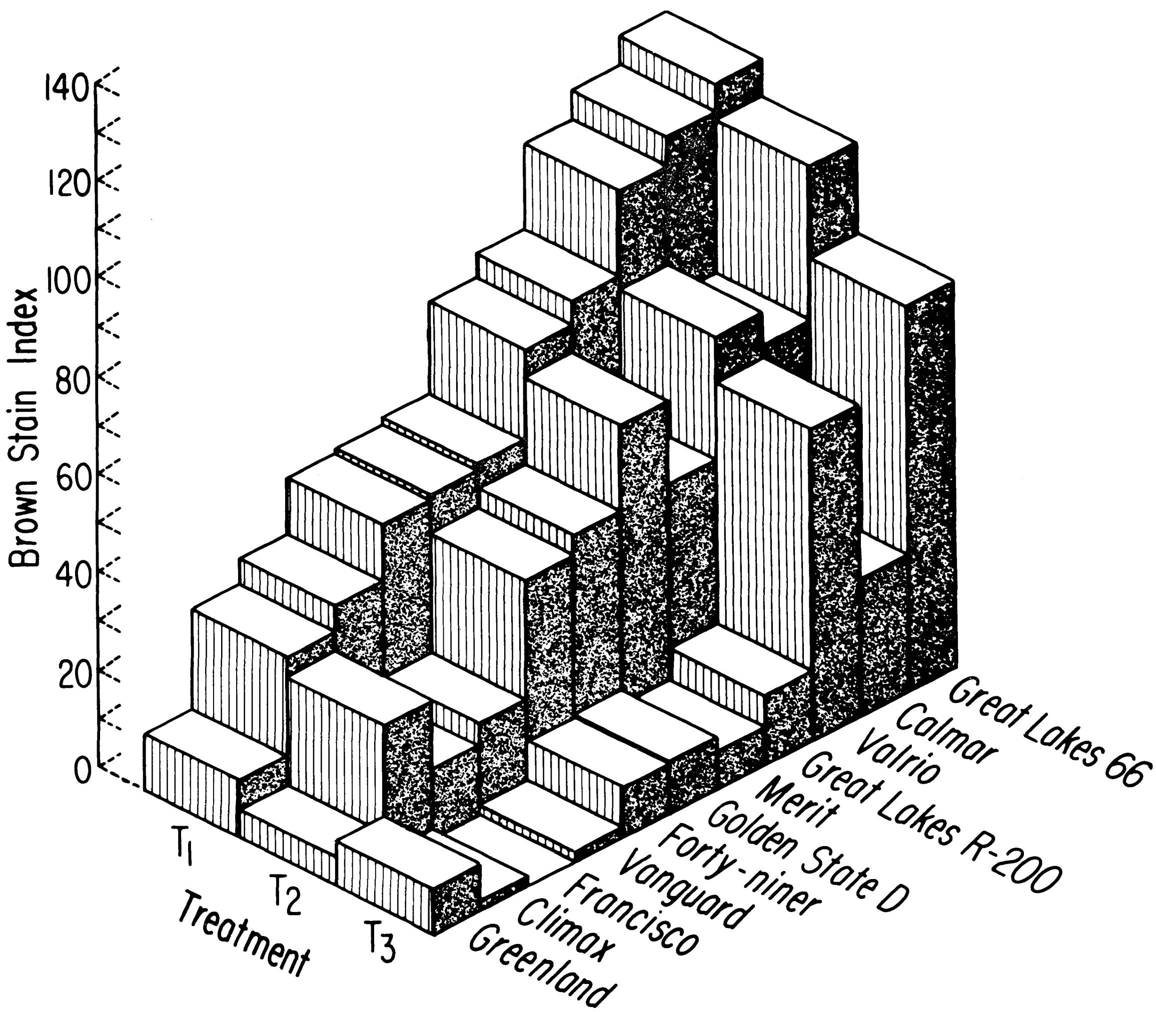

Fig. 1. Ternary histogram presenting lettuce cultivar susceptibility to brown stain inducing treatments. Data shown represent the mean index of all replicates for a given treatment-cultivar combination. T_1 = 2% O_2, 10% CO_2, 2.5°C; T_2 = 2% O_2, 5% CO_2, 0°C; T_3 = 2% O_2, 5% CO_2, 2.5°C. See text and Table 1 for details.

susceptibility to brown stain and there were treatment-cultivar interactions. Aside from severity, the symptoms were similar for all cultivars. Table 1 permits a comparison of cultivars within the individual tests; the LSD values for the 5% level are given. The average for each cultivar for the tests that include that cultivar is given in Table 1 and values are plotted in Fig. 1. In comparing cultivar averages, it must be kept in mind that they represent a variable number of tests.

The cultivars showed a wide range of susceptibility but they could not be grouped accurately due to variability among tests and interaction with treatment. Considering all tests and all treatments, one can generalize that 'Great Lakes 66', 'Calmar', 'Valrio', 'Merit' and 'Great Lakes R-200' showed more susceptibility than other cultivars. Using 3 groupings the following seems justified: 1) most susceptible - 'Great Lakes 66', 'Calmar', and 'Valrio'; 2) intermediate - 'Great Lakes R-200', 'Merit', 'Golden State D', 'Forty-niner', and 'Vanguard'; and 3) least susceptible - 'Francisco', 'Climax', and 'Greenland'. Large and consistent differences in cultivar susceptibility may be seen by comparing 'Calmar' (4 tests) and 'Climax' (5 tests). Following T_1, 'Calmar' showed severe to very severe injury whereas 'Climax' showed slight to moderate injury and comparable differences existed for T_2 and T_3. The relative susceptibility of the cultivars as reported here is in general agreement with the results of Stewart and Matoba (1972).

We suggest that cultivar differences in brown stain susceptibility be given consideration under commercial conditions and in programs of cultivar improvement and testing. In research related to CA effects on lettuce it is essential to consider and report the cultivars studied. It would be of interest to determine whether the cultivar differences are due to inherent differences in susceptibility to CO_2 injury or to differences in symptom development.

The variability among tests for a given cultivar indicate pretreatment variables not yet identified. Under investigation are: growing conditions, temp during harvest period, and time delays between harvest and treatment. Differences in head maturity are not likely to be the cause of the observed differences in brown stain. First, this factor does not appear to be important and, second, it was kept relatively uniform in these tests.

From the standpoint of reduced brown stain, transit temp somewhat above $0^{\circ}C$ could be desirable. However, it seems best to eliminate the causal factor (CO_2) and retain the benefits of the lowest safe temp obtainable. Thus accumulation of CO_2 during transit should be avoided (Watada et al., 1964).

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