Saline Aerosol: Some Effects on the Physiology of *Phaseolus vulgaris*

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ABSTRACT

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Experiments were performed to determine some of the chemical and physiological changes accompanying exposure of bean plants (*Phaseolus vulgaris* 'Topcrop') to saline aerosol. Plants were exposed to various dosages of salt (0–150 μg Cl^-/cm^2) when the primary leaves were approximately one-quarter expanded (7–8 days old). Respiration, photosynthesis, and transpiration rates were determined after salt exposure. There was an increase in the respiration rate of salted plants as compared to the unsalted controls. Photosynthesis apparently was not affected by saline aerosol when the rate of O_2 evolution was expressed on an area or a dry weight basis; however, the rate increased when expressed on a unit chlorophyll basis. Transpiration rate decreased with exposure to saline aerosol. When the primary leaves were fully expanded (15–17 days old) they were analyzed for contents of chloride, water, total nitrogen, total chlorophyll, total free amino acids, soluble sugar, and starch. The chloride content increased linearly with increased exposure. Water content per unit area or per unit fresh weight increased upon exposure to salt although the relative turgidity of the tissue did not change. As the chloride content increased, the total nitrogen content decreased. Chlorophyll and amino acid contents increased until symptoms appeared, then they decreased. With increased exposure to salt total soluble sugar content increased, but there was no significant change in the starch content of the leaves.

Additional key words: saline spray, physiological effects.

The recent development of evaporative saltwater cooling towers to dissipate thermal pollution from energy generating plants will involve saline drift (11,21). Airborne salt has detrimental effects on plants (2). Plants growing in coastal areas (7) or along roadways deiced with salt (9,16–18) often display foliar injury attributable to wind-driven salt spray.

Airborne salt injury in nature has been simulated under controlled conditions (8,20,22). Although a considerable amount of work has been done on the physiological effects of substrate salinity on plants (3,4,12–14,19), few detailed studies have been reported on the effects of salt applied to foliage (8,22). The purpose of this study is to investigate some of the more subtle effects of airborne salt on *Phaseolus vulgaris*.

MATERIALS AND METHODS

Cultural methods. All experiments were performed with primary leaves of bush bean (*Phaseolus vulgaris* L. 'Topcrop'). Seeds were sown in vermiculite and transplanted (1/7.6 cm pot) into a mixture of soil, peat, and perlite (1:1:1, v/v) when the primary leaves were just starting to emerge (5–6 days old). The plants were placed on a greenhouse bench and allowed to grow until the primary leaves were approximately one-quarter expanded (7–10 days old), at which time they were selected for uniformity and exposed to saline drift in specially designed sedimentation chambers.

Exposure methods. The sedimentation chamber consisted of a wooden frame (1m × 1m × 1.5m high) covered with polyethylene and arranged with a resealable flap to allow entrance. Salt mist was generated from a synthetic seawater solution by a spinning disk humidifier placed below the chamber. The aerosols were forced upward in a 1-m high, 10-cm diameter PVC column in the center of the chamber and out through holes in the top. The fine droplets of salt solution then settled to the bottom of the chamber. Plants could be placed in the chamber and exposed to various dosages of saline aerosol. Salt output was determined by placing open petri plates in the bottom of the chamber and measuring the salt collected. Salt dosage was expressed as micrograms of chloride per unit area.

When primary leaves were approximately one-quarter expanded, plants were placed in the sedimentation chamber and exposed to various dosages of saline aerosol. During exposure, the temperature was maintained at 25–30 C and the relative humidity, at 75–85%. After exposure, the plants were removed from the chamber and returned to the greenhouse bench.

Analytical methods. Leaves were allowed to expand fully (15–18 days old) at which time they were harvested, rinsed with deionized water, and dried at 50 C in a forced-air oven. The dried tissue was ground to a fine powder with a mortar and pestle. Samples (100 mg) of the dried tissue were analyzed for contents of chloride, total nitrogen, total chlorophyll, soluble sugar, starch, and free amino acids.

Chloride was extracted in deionized water and analyzed by means of a Buchler-Cotlove chloridometer. Chloride content was expressed as percent of dry weight.

TABLE 1. Effect of saline aerosol on the total nitrogen and total chlorophyll contents of *Phaseolus vulgaris*[y]

Salt level (μg Cl^-/cm^2)	Chloride (% dry wt)	Nitrogen (% dry wt)	Chlorophyll (mg/g dry wt)
0	0.51 a	4.94 a	8.8 a
45	1.35 b	4.57 ab	9.1 a
91	2.35 c	4.45 b	10.4 b
170[z]	3.17 d	4.19 b	8.4 a
LSD @ $P = 0.05$	0.38	0.38	0.9

[y]Each value is the mean of 10 replicates. Numbers in a column followed by the same letter are not significantly different, $P = 0.05$.
[z]Plants had symptoms.

TABLE 2. Effect of saline aerosol on the total free amino acid content of *Phaseolus vulgaris*[y]

Salt level (μg Cl^-/cm^2)	Chloride (% dry wt)	Amino acid (mg/g dry wt)
0	0.45 a	1.9 ab
20	0.97 b	2.0 ab
58	1.35 c	2.6 b
94[z]	2.52 d	1.5 a
LSD @ $P = 0.05$	0.27	1.0

[y]Each value is the mean of five replicates. Numbers in a column followed by the same letter are not significantly different, $P = 0.05$.
[z]Plants had symptoms.

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Tissue samples were analyzed for total nitrogen by the micro-Kjeldahl wet-digestion (15). Nitrogen content was expressed as percent of dry weight.

Chlorophyll content was determined spectrophotometrically by the method of Arnon (1) and expressed as milligrams of chlorophyll per gram of dry weight.

Soluble sugars were extracted in ethanol and analyzed by the anthrone colorimetric method (24). Sugar content was expressed as milligrams of sugar per gram of dry weight. The tissue that had been freed of soluble sugars was resuspended in deionized water and heated to gelatinize the starch. The starch was treated with perchloric acid to break it down into glucose (6) and the glucose concentration was determined by the anthrone colorimetric method (24). Starch content was expressed as milligrams of glucose released per gram of dry weight.

Amino acid content was determined by the ninhydrin colorimetric method (23) and expressed as milligrams of amino acid per gram of dry weight.

Physiological methods. After exposure to saline aerosol the leaves were allowed to fully expand, and leaf disks were cut randomly and weighed fresh. They were then floated on deionized water for 1 hr, blotted dry and reweighed. The disks were then dried overnight in a forced-air oven at 50 C and weighed again. Water content, expressed as total water content (TWC), percent water content (PWC) and relative water content (RWC, an estimation of the relative turgidity of the tissue), was calculated in the following manner:

$$TWC = \frac{\text{fresh weight} - \text{dry weight}}{\text{area of disks}} = \text{mg } H_2O/\text{cm}^2$$

$$PWC = \frac{\text{fresh weight} - \text{dry weight} \times 100}{\text{fresh weight}} = \text{mg } H_2O/100 \text{ mg fr. wt.}$$

$$RWC = \frac{\text{fresh weight} - \text{dry weight} \times 100}{\text{turgid weight} - \text{dry weight}} = \%$$

Transpiration rates were determined with a diffusive resistance meter. Rates were expressed as percent of controls.

Photosynthesis and respiration rates were measured by oxygen evolution or uptake in a Gilson differential respirometer. Rates of oxygen uptake or evolution were expressed per unit dry weight, per unit area and, in the case of photosynthesis, per unit chlorophyll.

Analysis of variance was performed on all data. The least significant difference multiple range test was used to compare treatment means.

TABLE 3. Effect of saline aerosol on the soluble sugar and total starch contents of *Phaseolus vulgaris*[y]

Salt level (μg Cl^-/cm^2)	Chloride (% dry wt)	Sugar (mg/g dry wt)	Starch (mg glu/g dry wt)
0	0.55 a	26.4 a	35.1 a
49	1.51 b	29.0 ab	35.0 a
92	2.25 c	31.8 b	35.0 a
144[z]	3.72 d	38.4 c	34.3 a
LSD @ $P = 0.05$	0.24	4.1	2.7

[y]Each value is the mean of 10 replicates. Numbers in a column followed by the same letter are not significantly different, $P = 0.05$.
[z]Plants had symptoms.

TABLE 4. Effect of various levels of saline aerosol on the rate of apparent photosynthesis of *Phaseolus vulgaris*

Salt level (μg Cl^-/cm^2)	Rate of photosynthesis[y] (μl $O_2/cm^2/hr$)	(μl O_2/mg dry wt/hr)	(μl O_2/μg Chl/hr)
0	51.9 a	22.8 a	2.1 a
51	53.4 a	19.6 a	2.5 b
101[z]	54.7 a	22.9 a	2.7 b
146[z]	54.9 a	24.3 a	3.2 c
LSD @ $P = 0.05$	12.6	5.1	0.3

[y]Each value is the mean of four replicates. Numbers in a column followed by the same letter are not significantly different, $P = 0.05$.
[z]Plants had symptoms.

RESULTS

Effect of saline aerosol on the chemical composition of *P. vulgaris*. In all experiments, chloride content of the tissue increased linearly with increased exposure to saline aerosol. Symptoms of chlorosis usually appeared when the chloride content reached approximately 3% of the foliage dry weight (Tables 1, 2, 3, 6).

Saline aerosol had a marked effect on the total nitrogen content of bean leaf tissue (Table 1). Plants exposed to the higher (90 or 170 μg Cl^-/cm^2) levels of salt contained significantly less foliar nitrogen when the content was expressed on a dry weight basis than did the unsalted controls. This nitrogen decrease occurred even in the absence of symptoms, which became apparent at the 170 μg Cl^-/cm^2 level.

Total free amino acid content increased, though not significantly, with increasing exposure to salt until symptoms appeared (94 μg Cl^-/cm^2), when it decreased (Table 2). Chlorophyll content was significantly greater in leaves of plants exposed to 90 μg Cl^-/cm^2 than that of control plants (Table 1). Once symptoms became apparent (1,970 μg Cl^-/cm^2) the chlorophyll content decreased.

Soluble sugar and starch contents of salt-exposed plants are presented in Table 3. The sugar content of the leaf tissue was greater than that of the unsalted controls; however, starch content of the leaves was not significantly altered by exposure to saline aerosol.

Effect of saline aerosol on the rate of photosynthesis and respiration of *P. vulgaris*. Photosynthesis was not affected by exposure of plants to saline aerosol when the rate was expressed on an area or a dry weight basis, but was significantly greater in salt-exposed plants when expressed per unit chlorophyll (Table 4). Plants exposed to 101 or 145 μg Cl^-/cm^2 showed visible symptoms of chlorosis.

Respiration rate increased significantly at all levels of saline aerosol when expressed on a dry-weight basis, whether or not symptoms were apparent (Table 5).

TABLE 5. Effect of various levels of saline aerosol on the rate of foliar respiration of *Phaseolus vulgaris*

Salt level (μg Cl^-/cm^2)	Rate of respiration[y] (μl $O_2/cm_2/hr$)	(μl O_2/mg dry wt/hr)
0	5.0 a	2.1 a
51	6.2 a	2.7 b
101[z]	6.3 a	2.9 b
146[z]	6.7 a	2.8 b
LSD @ $P = 0.05$	2.5	0.5

[y]Each value is the mean of four replicates. Numbers in a column followed by the same letter are not significantly different, $P = 0.05$.
[z]Plants had symptoms.

TABLE 6. Effect of saline aerosol on total and relative water contents of *Phaseolus vulgaris*[v]

Salt level (μg Cl^-/cm^2)	Chloride (% dry wt)	TWC[w] (mg H_2O/cm^2)	PWC[x] (mg H_2O/100 mg)	RWC[y] (%)
0	0.49 a	15.2 a	84.5 a	85.1 a
60	1.92 b	16.0 ab	86.6 b	85.7 a
93	2.98 c	17.2 b	88.6 c	86.4 a
146[z]	4.06 d	17.1 b	88.9 c	83.5 a
LSD ($P = 0.05$)	0.45	1.8	0.9	3.5

[v]Each value is the mean of 10 replicates. Numbers in a column followed by the same letter are not significantly different, $P = 0.05$.
[w]Total water content.
[x]Percent water content.
[y]Relative water content.
[z]Plants had symptoms.

TABLE 7. Effect of saline aerosol on the transpiration rate[y] of *Phaseolus vulgaris*[z]

Salt dosage ($\mu g\ Cl^-/cm^2/day$)	Transpiration rate (Day 1) upper	(Day 1) lower	(Day 2) upper	(Day 2) lower	(Day 3) upper	(Day 3) lower
0	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a	1.00 a
50	0.78 b	0.99 a	0.63 b	1.10 a	0.39 c	0.81 b
100	0.62 c	0.96 a	0.51 c	1.00 a	0.56 b	0.53 c
LSD ($P = 0.05$)	0.10	0.10	0.10	0.10	0.10	0.10

[y]Transpiration rate is expressed as percent of controls.
[z]Each value is the mean of 10 replicates. Numbers in a column followed by the same letter are not significantly different, $P = 0.05$.

Effect of saline aerosol on water relations of *P. vulgaris*. Plants exposed to saline aerosol had a significant increase in total and percentage of water content, but relative water content did not change significantly upon exposure (Table 6).

Upper leaf surface transpiration rate decreased after the first day of exposure to saline aerosol (Table 7). By the second day of exposure the rate was half that of control plants. Lower leaf surface transpiration rate was not affected until the third day of exposure, when it too decreased. By the third day of exposure, plants were showing severe symptoms.

DISCUSSION

Airborne salt injury in plants arises from salt-induced alterations in metabolism. We investigated some of these changes. Bean plants, of a cultivar known to be sensitive to salt, were exposed to increasing dosages of saline aerosol comparable to levels commonly experienced by plants growing in coastal areas, until symptoms of chlorosis appeared. Plants that received greater dosages of saline aerosol contained higher foliar levels of salt. Visible symptoms usually developed when the chloride content reached approximately 3% of the dry weight.

Alterations in the chemical composition of primary leaf tissue exposed to saline aerosol indicated that elevated levels of salt in the tissue affected the metabolic activities of bean plants prior to the appearance of injury symptoms. The decrease in total nitrogen is consistent with the results of Gauch and Wadleigh (4) who also found decreased total nitrogen content in bean plants grown in saline media. The accompanying increases in free amino acid and soluble sugar contents agree with the work of Gauch and Eaton (3), and of Strogonov (19). These results could indicate a salt-induced decrease in protein synthesis or an increase in protein degradation.

The chlorophyll content per unit dry weight increased prior to the appearance of injury symptoms. Nieman (12) observed similar results with bean plants grown in saline culture solutions. Once toxic levels of salt in the tissue were reached, symptoms of chlorosis appeared, suggesting either a salt-induced decrease in synthesis or a stimulation of chlorophyll breakdown.

Photosynthesis was apparently not affected by saline aerosol when the rate of O_2 evolution was expressed on an area or a dry weight basis, even after symptoms of chlorosis appeared. The lack of any effect on the rate of O_2 evolution, together with the accumulation of soluble sugars, indicates that the salt is interfering with the utilization of carbohydrate rather than with photosynthetic activity.

The increased O_2 uptake by plants exposed to saline aerosol is consistent with the work of Nieman (12) who observed increased respiration in bean plants grown in saline culture media. Because the respiratory activity of isolated mitochondria depends on the ionic environment of the media (5), and because saline aerosol affects the ionic composition of leaf tissue, it is possible that mitochondria may be affected in such a way as to lead to increased respiratory activity.

Increased tissue succulence is a common plant response to salinity (2,4,12). In the present experiments, plants exposed to saline aerosol exhibited a similar increase in water content when it was expressed on an area or weight basis. However, the relative water content did not change upon exposure to salt. Because the tissues of plants exposed to saline aerosol contained higher levels of salt, the tissues may have had to absorb more water to dilute the excess salt and thus to maintain the same water potential.

Transpiration rate decreased upon exposure of plants to saline aerosol. Meiri and Poljakoff-Mayber (10) also observed decreased transpiration rate in bean plants growing in saline media and attributed it to the decrease in the diffusion-pressure gradient between media and plant. With airborne salt the situation is different. The effect is probably directly on the guard cells.

Levels of saline aerosol used in the present studies covered the range of deposition rates commonly found in seashore areas and those expected around saltwater cooling towers. Deposition rates along the New Jersey coast range from approximately 400 $\mu g/cm^2/day$ at the edge of the surf to 20 $\mu g/cm^2/day$ 600 m inland (22). Deposition rates as high as 100 $\mu g/cm^2/day$ have been observed 100 m from a saltwater cooling tower in Texas (21). Such levels were high enough to cause significant increases in salt content of bean leaf tissue resulting in metabolic alterations.

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