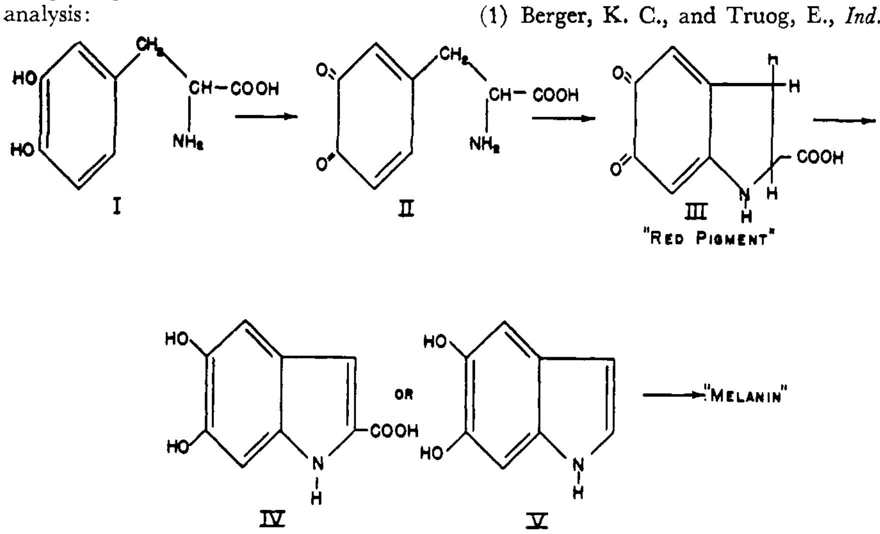
ment formed in one half the time. After boron was added to the 3,4-dihydroxyphenylalanine–azotobacter mixture, a change was noted in the conversion of 3,4-dihydroxyphenylalanine. The final color produced was a red-brown pigment which did not change upon further incubation. It is evident from these tests that boron has a definite influence in the conversion of melanin precursors to melanin.

The various data have shown that the presence of boron, copper, and specific amino acids causes increasing pigmentation of azotobacter, with boron causing the production of a definitely redder pigment in the basal medium. This indicates that boron inhibits the complete oxidation of pigment precursors to melanin. The inhibitive action evidently occurs after hallochrome formation (red pigment, III), according to the following scheme of Raper (*16*), which has been substantiated by Mason (*12*) by spectrophotometric and manometric analysis:



The effect of copper both in the basal medium and in the 3,4-dihydroxyphenylalanine solution was to influence the rapid formation of a dark brown to black melanoid pigment. This indicates that the above scheme was not inhibited but enhanced by the presence of copper. In the experiments where boron and copper were combined, a moderate amount of boron (14 to 70 p.p.m.) did not greatly inhibit the oxidation of the pigment precursors to melanin. However, at higher ranges of boron concentration the pigment-producing ability of the accompanying copper was restricted and frequently no appreciable pigmentation was noted. These results suggest that the presence of boron in higher amounts blocks the activity of copper by influencing the enzymatic activity in a direction which produces a substance that is not readily and completely oxidized to melanin even in the presence of copper.

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AIR POLLUTION EFFECTS

Lime Papers and Indicator Plants in Fluorine Air Pollution Investigations

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Industrialization has increased the possibility of air contamination from effluent fluoride in a number of areas of the state of Washington. This paper reports results of a search for inexpensive methods that may be used to detect atmospheric fluoride and to delineate areas where it may be an economic factor with respect to farming. The use of gladiolus and lime-treated filter paper is described as a method of estimating areas where sufficient fluorides are in the air to increase the fluoride content of forage used for cattle pasture.

The increasing industrialization of the state of Washington as well as other parts of the nation has resulted in the problem of air pollution damage to agricultural crops. As there are at least five locations in the state of Washington where there are possible atmospheric fluoric effluents, an inexpensive and reliable method of estimating damage areas is needed. The agricultural interests involved in these areas in this state include dairy and beef cattle, gladiolus plantings, and Italian prune and other orchards.

Adams *et al.* (*1*) described an air-analysis system used at Spokane, Wash., in the investigation of pine tree damage. However, this method required the continuous time of several men to tend the air-absorption apparatus. MacIntire *et al.* (*5*) used Spanish moss as a means of absorbing atmospheric fluoride. Compton and Remmert (*3*) have used potted buckwheat plants in a somewhat similar manner. Ost (*6*) hung cloths treated with limewater in trees for 5 to 7 months. In each case the investigators determined fluoride in the absorbing medium. The present communication is a report of the use of lime-treated filter paper and gladiolus plants to estimate areas where fluoric effluents may be a factor.

Materials and Methods

The Ethel Cave Cole variety of gladiolus was used, because it has been shown that in this variety visible leaf injury from atmospheric fluoride is readily observable (*4*). Six large gladiolus corms were planted in uniform soil in clean 5-gallon buckets. The soil level was 5 to 7 cm. below the rim of the buckets, so that watering of the plants in the dry season was accomplished by filling with water. The gladiolus were started in an uncontaminated locale until the plants were about 15 cm. high. On May 26, 1952, the buckets were taken to the field and placed in the ground so that the soil in the buckets was approximately level with the surrounding soil. The plants were protected from insect damage by covering with a cylindrical screen approximately 90 cm. high and 30 cm. in diameter. It was necessary to water the plants approximately every 2 weeks in western Washington. On September 15 and 16 the injury index was determined (*4*). This 16-week exposure period corresponded to four lime paper exposure periods. The tip 75 mm. of the leaves were collected for chemical analysis.

Whatman No. 1 filter paper, 12.5-cm. size, was dipped in lime suspension (28 grams per liter of low-fluorine lime), hung on a glass rack, and dried in an oven at 50° C. The papers were exposed in groups of six at the various locations in shelters which allowed air movement, but protected the papers from the weather (Figure 1). The boxes were supported by stakes about 1.8 meters off the gound, and were located away from roads or other sources of dust. The filter papers were fastened top and bottom by clothespins to cross wires in a rack. The wires were spaced 25 mm. apart horizontally and 117 mm. apart vertically. Following preliminary investigations in 1950 and 1951, exposure periods of 4 weeks were used in 1952. Forage samples were taken at the same time as the lime paper samples, and in fields adjacent to the lime paper shelters where cattle were grazing or had recently grazed.

The samples for fluorine determinations were taken in two ways. In the first procedure, sufficient forage to give 30 to 40 grams of dry material was placed in a plastic container of 1500-ml. capacity. Three grams of low-fluorine lime were added and the friction top lid was placed on the container. The forage and lime were shaken vigorously to coat the forage thoroughly with lime. Then the container was returned to the laboratory, the lid was removed, and the forage was dried at 50° C. for 2 days. The sample was then ground in a Wiley mill. In calculation of the amount of fluorine, correction was made for the lime added. The other procedure for the taking of samples was to clip the forage, place it in an ordinary cylindrical freezing carton, tape on the lid, and then store at −18° C. until analyzed. At time of analysis, the sample was ground through a food chopper in the frozen condition, and a suitable portion taken for determination of fluorine and dry matter. Analyses of duplicate samples taken by both procedures showed essentially no difference in fluorine content, nor did 90 days' storage in the frozen condition change the fluorine value obtained (Table I).

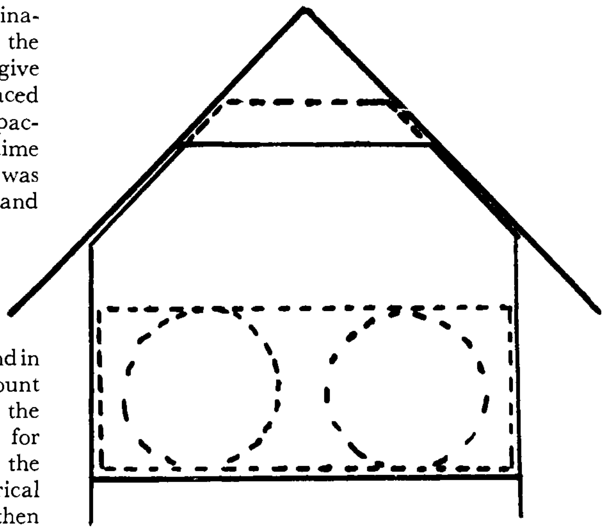


Figure 1. Diagram of box containing lime papers

Air circulation is through 0.5-inch hardware cloth bottom and 6.5-cm. openings under eaves. Dotted circles represent lime papers. Construcion is of $^{3}/_{8}$-inch plywood. Scale, 1 to 1.6

Table I. Effect of Method of Sample Treatment on Fluoride in Forage

(Values in p.p.m. of fluorine)

Immediate Lime Treated	Less Than 5-Day Storage at −18° C.	90-Day Storage at −18° C.
7	6	4
18	16	16
6	7	7
7	7	7
44	51	51
	55	50
	13	14
	404	415

Fluorine was determined in forage by the sodium hydroxide fusion method reported by Remmert *et al.* (*7*) with minor modifications. The filter paper and gladiolus leaf samples were ashed and distilled by a modified AOAC procedure (*2*).

The titration was a modification of the method of Smith and Gardner (*8*). An aliquot of the distillate not exceeding 85 ml. in volume nor containing more than 30 γ of fluorine was measured into a 200-ml. tall-form beaker. Five milliliters of 5 *N* sodium chloride were added, followed by 1 ml. of a 1% solution of hydroxylamine hydrochloride. The beaker was swirled, and 2 ml. of a solution of sodium alizarin sulfonate (25 mg. of the indicator and 32.5 ml. of 0.5 *N* hydrochloric acid in 250 ml. of water) were added. The pH of the solution was adjusted to exactly 2.85 on a Beckman pH meter by the addition, if necessary, of small amounts of 0.05 *N* hydrochloric acid or sodium hydroxide. The sample was titrated in a 100-ml. Nessler tube by addition of small increments of the thorium nitrate reagent (0.134 gram of thorium nitrate tetrahydrate plus 15.0 ml. of 0.5 *N* hydrochloric acid per liter) to the sample tube with mixing by inversion between additions. The end point was reached when the color of the sample tube matched the permanent standard. The stock solution of the permanent color standard was prepared by mixing 52 ml. of 3.66% cobalt chloride hexahydrate, 6.0 ml. of 0.65 *N* hydrochloric acid, and 13.2 ml. of 0.1% potassium chromate, and diluting to 100 ml. The working permanent color standard was prepared by dilution of 5 ml. of the stock solution to 100 ml. in a tall-form Nessler tube. The thorium nitrate reagent was standardized by titration of known amounts of fluorine.

Results and Discussion

The exposure boxes were of sufficient size to contain several groups of papers.

Table II. Fluoride Content of Papers in Different Locations in Exposure Box

(Values in p.p.m. of fluorine)

Group 1	Group 2	Group 3
49	46	. .
44	42	. .
15	17	. .
18	20	22
13	13	. .
7	8	. .
18	18	. .
42	44	. .
28	28	. .
19	20	21

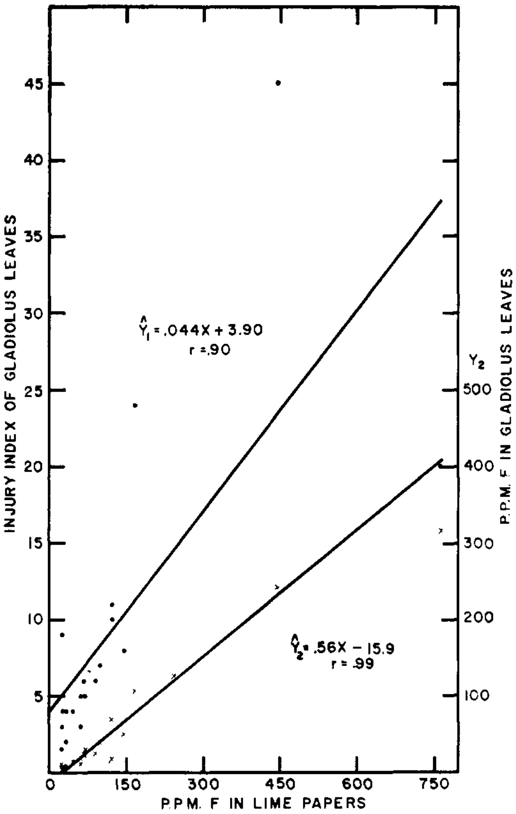


Figure 2. Regression of fluorine in gladiolus leaves and injury index of gladiolus leaves on fluorine in lime papers

The data in Table II show that only small differences in fluorine content may be expected among groups of six papers when exposed for 4 weeks in different locations in the same box. In two cases, three groups of papers were exposed; in the others only two.

To investigate the cumulative absorption of fluoride by the papers, one group was exposed 8 weeks and the results were compared with the sum of the corresponding two 4-week exposures (Table III). The values found indicate that the average of the 8-week exposures is 93% of two successive 4-week exposures. The sum of the fluorine absorbed by the papers during two 4-week periods would not be less than 84% or more than 119% of the amount absorbed in 8 weeks.

Table III. Exposure Time to Uptake of Fluorine in Lime Papers

Sum of Two 4-Week Periods, P.P.M. F	*Corresponding 8-Week Period, P.P.M. F*
325	279
83	90
368	361
114	136
30	28
69	79
6	5
69	68
13	12
19	17
20	19
22	23
38	33
146	119
310	252
157	147
Av. 112	104

To determine the value in estimation of locales where air is polluted by fluorine the results obtained by determination of fluorine in the lime papers and gladiolus and the injury index obtained from the gladiolus were compared with the analyses of forage from adjacent fields. The forage analyses from each of three areas of western Washington where atmospheric fluoride may be present were correlated statistically with lime paper and gladiolus fluoride and with gladiolus injury index.

Both linear and curvilinear regressions were calculated. In all cases the curvilinear regressions improved the correlations only very slightly. Only the linear regressions are presented here, and are summarized in Table IV.

The regression of parts per million of fluorine in gladiolus leaves and the regression of the injury index of the gladiolus leaves on the parts per million of fluorine in the lime papers are presented in Figure 2. The values for the lime papers in these comparisons are the total for four exposures of 4 weeks each, a period which coincided with the exposure of the gladiolus. The correlation between the fluorine content of the lime papers and the fluorine content of the gladiolus leaves is extremely high, as indicated by the r value of 0.99. It is apparent that the lime papers were approximately twice as effective in absorbing fluorine as were the gladiolus leaves. While the correlation was not as good between the injury index of the gladiolus leaves and the fluorine content of the lime papers, the r value of 0.90 is above that required for significance at the 1% level. From these data, it is apparent that the fluorine content of the gladiolus or the injury index can be predicted with a high degree of accuracy from the fluorine content of the lime papers.

Table IV. Correlation between Fluorine in Lime Papers and in Forages and Gladiolus, and Injury Index of Gladiolus

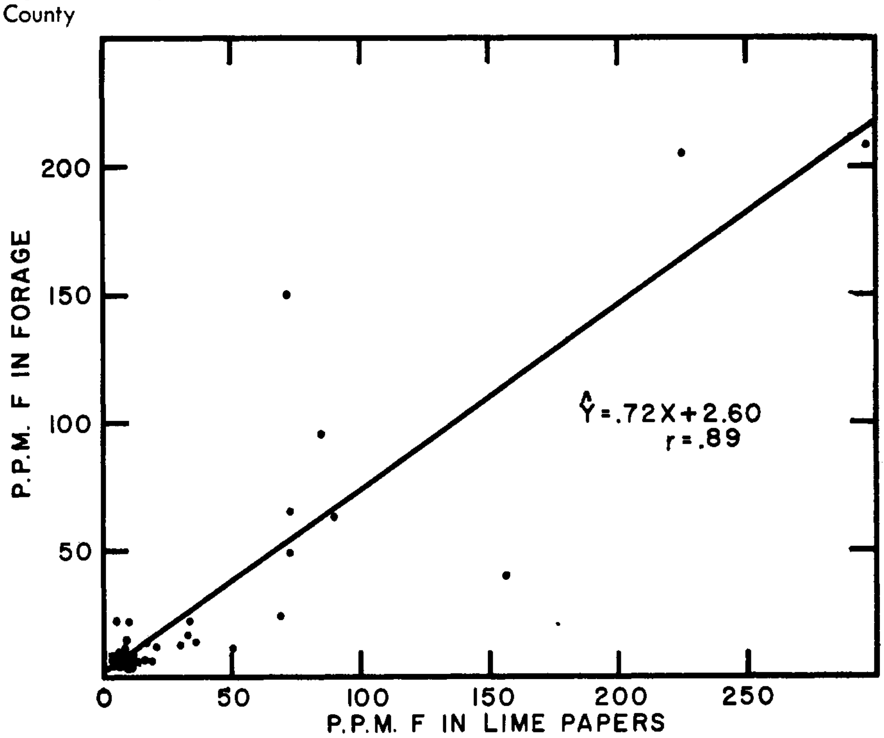
	n	r^a
Fluorine in lime papers, p.p.m.		
× F in gladiolus leaves, p.p.m.	21	0.99
× injury index of gladiolus	21	0.90
× F in forage, p.p.m.		
Cowlitz County	49	0.89
Pierce County	28	0.85
Clark County	63	0.49
All data	140	0.88

[a] $P < 0.01$.

The regression of parts per million of fluorine in forage on the parts per million of fluorine in the lime papers, both taken at 4-week intervals over a period of approximately 7 months for each of three areas involved, is shown in Figures 3, 4, and 5.

The r values in each comparison are above that required for statistical significance at the 1% level (*9*). The Cowlitz County data (Figure 3) and the Pierce County data (Figure 4) show better correlations than the data for Clark County (Figure 5), possibly because the fluorine values for both the lime papers and the forages were much lower in Clark than in the other two counties. This may indicate that the reliability in

Figure 3. Regression of fluorine in forage on fluorine in lime papers in Cowlitz County



estimating forage fluoride values may not be so accurate when the values are less than 20 p.p.m. as when the values are higher. The values for the lime papers generally were somewhat higher than the corresponding values for the forages. The poorer correlation between fluoride in lime paper and forage than between lime paper and gladiolus leaves may be due to the heterogeneous nature of the forage in a pasture. Also the forage was frequently being grazed by cattle, whereas the gladiolus leaf was exposed throughout the entire period.

The gladiolus injury index is the simplest way to estimate a contaminated area, because it does not involve chemical analysis. The growing season of the gladiolus does represent a substantial portion of the growing season for forage in western Washington. The sampling of forage to determine an area of air fluoride contamination is open to criticism. The forage sampled may not represent what cattle consume. A cow can, and frequently does, graze much closer than it is practical to clip forage, especially on some types of pasture.

The gladiolus or lime paper reacts to atmospheric fluoride, and, in effect, a record of the entire time of exposure is obtained. This method of investigation may prove of value in locales where a uniform coverage of an area is desirable. In communities where numerous types of farms are present, it is not always possible to find suitable vegetation at points where samples should be taken. This method may also be of value in towns or in areas where there is no suitable vegetation growing, such as hilly places or waste land. As the boxes are easily constructed and require little attention, they might be used in considerable numbers to follow air currents carrying fluoride. The method may be used on a year-round basis to detect atmospheric fluoride when no vegetation is available for sampling.

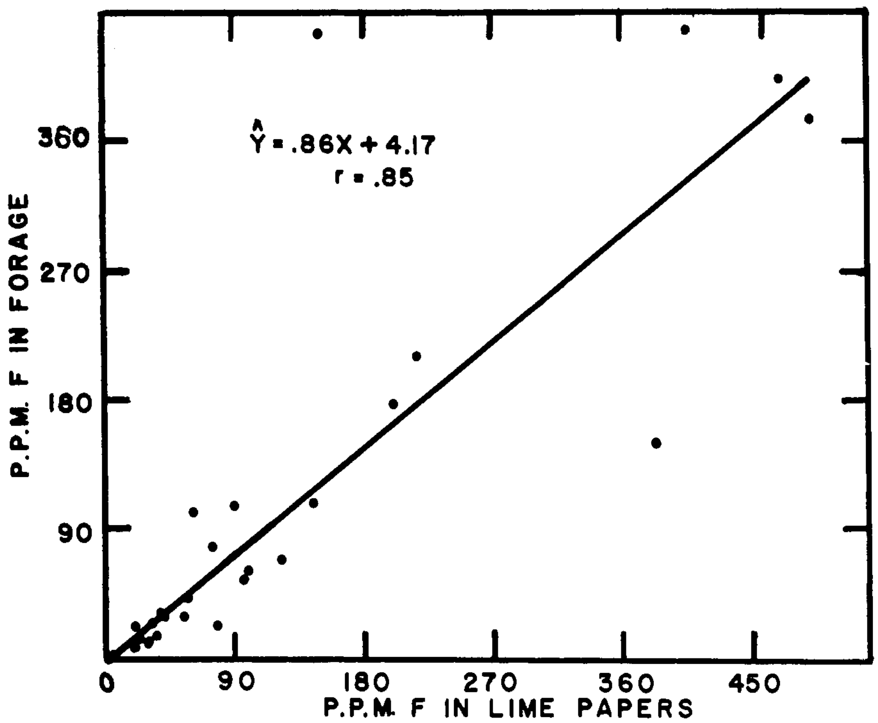


Figure 4. Regression of fluorine in forage on fluorine in lime papers in Pierce County

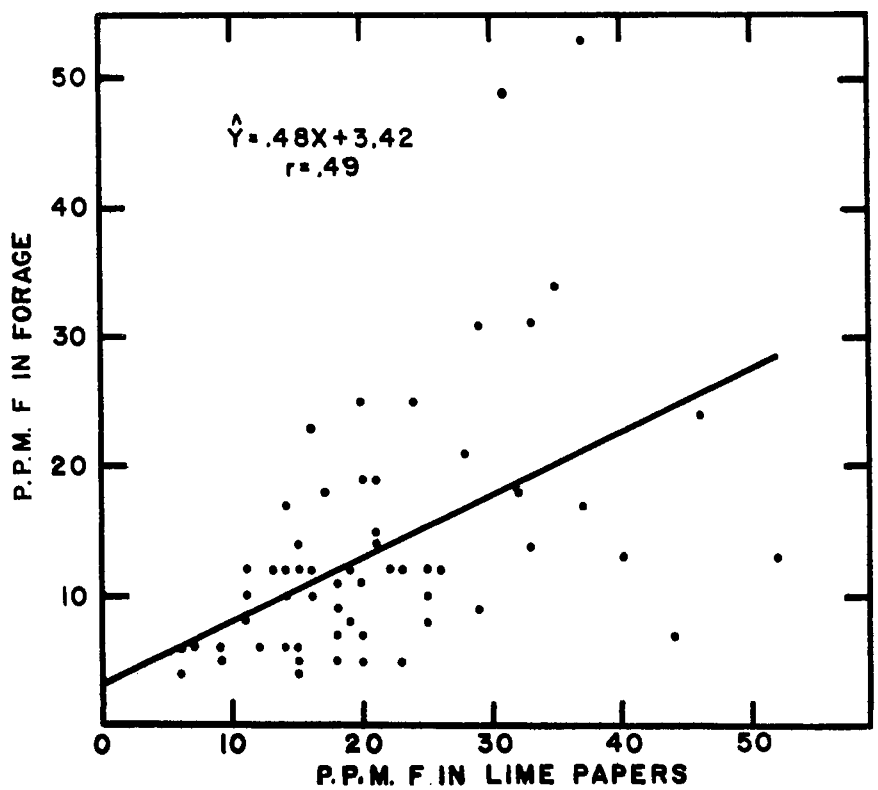


Figure 5. Regression of fluorine in forage on fluorine in lime papers in Clark County

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