CHAPTER 8

Some Problems Relating to Fluorides in the Environment: Effects on Plants and Animals

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Abstract

Several aspects of fluorides in the environment have been researched for decades so there is a great deal of published information. However, the authors consider that there are several important areas where information is lacking, data are contradictory, mechanisms of action have not been explored, or the environmental effects of particular compounds are not known. Therefore, this chapter reviews a selection of such topics: inorganic fluorides in soil; the loss of fluorides from plants; classifying species sensitivity to HF; estimating effects of fluorides on growth and yield; the significance of pollutant interactions; effects of HF on fertilization and seed set; fluorides and insects; and old and new problems associated with organofluorides in the environment.

1. INTRODUCTION

In Weinstein and Davison [1] we reviewed knowledge of all the major aspects of fluorides in the environment. During our research and writing the review it

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became clear that although many topics are well known and thoroughly researched, there are several important areas where information is lacking, data are contradictory, mechanisms of action have not been explored, or the environmental effects of particular compounds are not known. Therefore in this chapter we present a discussion of a selection of such topics in the hope that it will generate further interest: inorganic fluorides in soil; the loss of fluorides from plants; classifying species sensitivity to HF; estimating effects of fluorides on growth and yield; the significance of pollutant interactions; effects of HF on fertilization and seed set; fluorides and insects; and old and new problems associated with organofluorides in the environment.

2. QUESTIONS RELATING TO INORGANIC FLUORIDE IN SOIL

Soil is a source of fluoride for plants and animals, and it acts as a sink for atmospheric deposition so it plays a central role in the biogeochemical cycling of the element. Fluorine was first detected in minerals in the late 18th century and measured in a variety of rocks in the 19th century [1]. More detailed research on soil fluoride started in the 1930s and 1940s because of concerns about the increasing use of fluoride-containing phosphate fertilizers and inorganic pesticides. It was thought that their use might increase the fluoride content of forage or crops to unacceptable levels. The result of the research is that there is a great deal known about the mineralogy of soil fluoride, concentrations in different soil types worldwide, and the chemical species present. However, there are still important gaps in our knowledge such as the bioavailability of fluoride to plants and the chemistry and mobility of fluoride in organic horizons.

In the mineral horizons of soils, the fluoride concentration ranges from under 100 to several thousand mg kg^{-1} dry wt. [2,3]. In many regions the average is from about 100 to 600 mg kg^{-1}. Because soil fluoride is associated with clay-sized minerals, heavier soils tend to have substantially higher concentrations than do sandy soils. Table 1 shows some comparisons between sandy and clay soils. Concentrations higher than a few hundred mg kg^{-1} are found where there are deposits of high-fluoride minerals such as fluorspar and, in old mining areas, they

Table 1. Examples of the fluoride content of mineral soils in relation to sand and clay content

Author [Ref.]	Country	Sandy soils	Clay soils
Gemmell [9]	New Zealand	68	540
Nommik [10]	Sweden	43–198	248–657
Piotrowska and Wiacek [11]	Poland	20–150	250–750

may reach as high as 12–17% of the dry weight [4,5]. In humid climates there is usually a measurable increase in the fluoride content with depth due to leaching, provided the soil is not plowed and there is no significant deposition to the surface from the air or from fertilizers [6–8]. In situations where fluoride is added as aerial deposition or in fertilizer, there may be higher concentrations in the surface layers.

The leaves of plants growing on mineral soils that have a few hundred mg F kg^{-1} contain less than about 20 mg F kg^{-1}, and usually, under 10 mg F kg^{-1} [12,13]. This is because the bioavailability in most soils is low and because of the nature of plant roots. At soil pH above about 5.5, fluoride exists principally in the anionic F^- form [14] and when it diffuses passively into roots it is mostly confined to the extracellular region, the apoplast. This is probably because cell walls have fixed charges that promote exclusion of negatively charged F^- ions [15] and it limits the amount that is held at sites where it can pass the endodermis and into the conducting system. The endodermis around the conducting system has very low permeability to F^- so it acts as a barrier. Davison, Takmaz-Nisancioglu, and Bailey [16] and Takmaz-Nisancioglu and Davison [15] proposed that most of the fluoride that reaches the leaves leaks past the endodermal barrier at the root tips where it is poorly developed, and where lateral roots emerge. If this is correct, it suggests that the background fluoride content of leaves is related to water use so fluoride uptake may be higher in species with a high rate of water use. A highly branched root system may also promote greater uptake but this has not been investigated. This theory also explains why it is that when roots are exposed to concentrations of fluoride ions that are higher than normally occur in the soil solution, uptake is proportionately greater. Some data of Bar-Yosef and Rosenberg [17] support the idea that greater water use leads to higher fluoride uptake (Table 2). They grew corn (*Zea mays*) and tomato (*Solanum esculentum*) in hydroponic culture and measured the effects of increasing fluoride on growth,

Table 2. The rate of water use and fluoride content of corn and tomato exposed to different concentrations of fluoride in hydroponic culture (data from Bar-Yosef and Rosenberg [17])

Fluoride in solution (mg L^{-1})	Rate of water use (ml g^{-1} root day^{-1})		Fluoride content of shoots (mg kg^{-1})	
	Corn	Tomato	Corn	Tomato
0	8.9	13.7	7	5
1	7.9	13	8.1	7.2
5	7.4	13.1	8.4	11.2
10	7.9	12.9	12.4	28
50	7.4	12.4	54.5	126

water use, and fluoride content. As Table 2 shows, tomato used almost twice as much water as corn, and the fluoride content of the leaves was about twice as high at the elevated fluoride concentrations. Using hydroponic culture, leaf concentrations can reach a few thousand mg kg^{-1} and similarly, where soils are heavily contaminated with fluoride, such as mine wastes, leaf fluorides may be very high. For example, Cooke *et al*. [4] reported concentrations from 280 to over 4000 mg kg^{-1} in a range of grass and legume species growing on fluorspar waste in England.

The bioavailability of soil fluoride depends on the concentration in the solution and on the chemical species of fluoride present. This is an area of research in which considerable progress has been made in recent years, mostly by a group in Australia [14,18]. The minimum solubility occurs at pH values between about 5.5 and 6.5 [14,19]. This is the pH range of many of the most productive agricultural soils so the potential for significant fluoride uptake in those soils is minimal. However, the solubility and chemical speciation of fluoride change with pH. Research in Australia has shown that in soil, fluoride may exist as the free F^- ion or form soluble complexes such as SiF_6^{2-}, AlF^{2+}, AlF_2^+, AlF_3^0, AlF_4^-, BF_4^-, and HF [18]. Above about pH 6 the anion F^- predominates but the various complexes predominate at lower pHs. The significance of this difference is, as Stevens *et al.* [14] remarked, that the species of fluoride that are most readily taken up by plants, HF, and some of the aluminum complexes, exist at the pH values where it is most soluble. The permeability of cell membranes to HF and some of the aluminum complexes is much greater so they are more readily taken up than the anion F^-. Bioavailability increases steeply with a decrease in pH so plants growing on acid soils would be expected to have higher foliar fluoride concentrations than plants on neutral soils (Fig. 1). This should be most evident where there is contamination from aerial deposition, mine waste, or fertilizer. However, there is a further complication because acid soils usually have a significant organic content and that also affects fluoride bioavailability.

The fluorine chemistry and bioavailability of fluoride in humus or soil horizons with a high organic content have not been subjected to anything like the scrutiny of mineral soils. It is important to know more about this subject because of the ecological importance of decomposition processes and because a significant fraction of root systems may be in the organic layers. Also, many emission sources such as aluminum smelters are located in regions with well-developed organic horizons. The sources of fluorine in organic matter are plant residues, atmospheric deposition, and soil minerals. As indicated earlier, the background level of fluoride in vegetation is usually under 10 mg kg^{-1} so humus with no soil minerals incorporated into it and in an area with no atmospheric deposition would be expected to have a concentration similar to the vegetation, minus losses due to leaching. Deposition of dust on the surface or the incorporation of minerals by soil processes will increase the fluoride, so the concentration should, theoretically, be related to

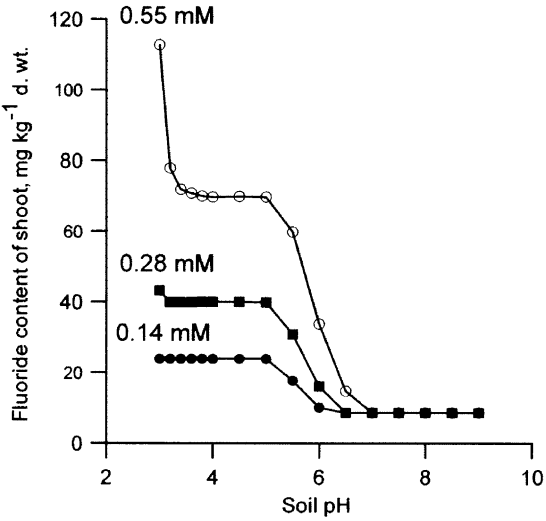

Fig. 1. Predicted shoot fluoride concentrations (mg F kg^{-1} day wt) in relation to soil pH and different concentrations of fluoride in solution: 0.14, 0.28, and 0.55 mM (2.65, 5.27, and 10.54 mg F L^{-1}, respectively). Drawn using data supplied by Stevens (personal communication) from a model described in Stevens *et al*. [14].

the mineral content. However, it is difficult to find published estimates of the total fluoride content of organic layers, especially in unpolluted regions. Thompson, Sidhu, and Roberts [20] reported 8–14 mg kg^{-1} fluoride in humus in an unpolluted area in Newfoundland while Omueti and Jones [7] found an average concentration of around 9 mg kg^{-1} in the organic matter from the surface layers of three Illinois sandy loams.

In situations where humus or other organic materials have a low mineral content the fluoride would be expected to be mobile because of the lack of fixation sites. This idea is supported by Pickering [21] who found that commercial humic acid retained only very low amounts of fluoride and even that was probably due to clay-like impurities rather than the humic acid itself. The low retention capacity of organic matter raises the question of what happens when the fluoride input is increased by atmospheric deposition. However, there are only a few studies that provide useful information, notably Thompson, Sidhu, and Roberts [20], Sidhu [22], and Polomski, Flühler, and Blaser [23]. The first two authors investigated the effects of atmospheric deposition from a phosphate factory. Sidhu [22] reported on the fluoride content of the litter of ten species growing at different distances from the factory, while Thompson, Sidhu, and Roberts [20] analyzed the humus in the same locations. Some of their data are given in Table 3. The litter data were collected in a different year from the rest so these have to be interpreted with

Table 3. The fluoride content (mg F kg^{-1} dry wt) in leaves, litter and humus of balsam fir (*Abies balsamea*) and the calcium content of the humus (data from Thompson, Sidhu, and Roberts [20] and Sidhu [22])

Zone in relation to F source	Average F content of foliage	Average F content of leaf litter	Average F content of humus	"Available" (= TISAB buffer extracted) F in humus	Calcium content of humus
I: 80–95% trees dead	281	892	908	58	14,800
II: 40–60% dead	141	272	205	27	7593
III: 20–30% dead	91	163	36	15	5810
IV: no dead trees, some light tip burn	44	75	16	3.8	2762
Control area	7	18	10	2.4	2150

caution but some points are still clear. Where there was high fluoride in the air, such as zone 1, the fluoride content of the litter was much higher than in the control area and this was reflected in the concentration in the humus. At first sight the data seem to suggest significant retention in the humus but there may be a special reason for this because there was a high rate of deposition of particulate materials containing calcium at this particular location (Table 3). However, the fluoride did not appear to be immobilized to the same extent as in mineral soils because Thompson, Sidhu, and Roberts [20] recorded that the humus fluoride decreased significantly during 1973–1974 even though emissions were 75% higher in 1974. They stated that this was due to "more efficient leaching … by groundwater." Clearly, further studies of fluoride retention and mobility in organic matter are warranted, particularly in areas where there is little calcium being deposited.

From a biological perspective it is important to be able to measure the fraction of soil fluoride that is available for uptake by plant roots. There have been many attempts to do this using a variety of techniques. Early studies mostly used a 1:1 soil–water extract to measure the "water–soluble" fluoride but others have used a saturated paste extract and many an electrolyte solution, acids, buffers, or complexing agents [24]. Larsen and Widdowson [19] used an anion exchange resin to extract the "labile" fluoride. Each of these extracts a different fraction to different extents and it is self-evident that a complexing buffer will extract different amounts than deionized water or an anion exchange resin. Many authors report their data as being the "available" fraction but the crucial test of an extraction

procedure is whether the results correlate with uptake by plants [25]. However, the degree of correlation varies greatly because Stevens *et al.* [26] found that published correlations give r^2 values ranging from 0 to 0.78. The reasons for this variability are discussed in Weinstein and Davison [1] but one of the most important results is that the amount available to roots depends on the fraction that is readily desorbed as the soil solution is depleted by the roots [19]. Also, the soil–solution ratio, the electrolytic composition, and the equilibration time all affect the amount of fluoride extracted from a soil. So, for example, if deionized water is used, the electrolyte concentration will vary with soil properties so it would be misleading to use this extractant to compare availability in organic and mineral horizons or leached sands with clays. Because soil pH affects solubility and speciation, if all of the soils examined have pH values above 5.5–6, the soluble fraction will tend to be universally low because of the dominating effects of pH over the total present. In that situation there is unlikely to be a correlation with plant uptake. Bioavailability and extraction procedures need to be re-examined to see if a more universally acceptable extraction procedure can be found that shows a high correlation with plant uptake over a wide range of soil pH and mineral/organic content.

It is generally concluded that in situations where plants are growing in the vicinity of a source of atmospheric fluoride, foliar fluoride concentrations will be dominated by direct uptake from the air and the contribution from the soil will be minimal [27]. However, there is very little evidence to confirm this in field situations where the total or ''available'' soil fluoride has been significantly increased by deposition. There are many reports of increased soil fluoride near atmospheric sources but in most cases it is impossible to tell if uptake from the soil is significant. However, three cases provide interesting data [28–30]. A comprehensive report on fluoride in relation to the Norwegian aluminum industry [28] contains an account of experiments in which plants were grown in fluoride-contaminated soil in areas where there was no atmospheric source. St John's wort (*Hypericum perforatum*) had only low levels of fluoride on the contaminated soils (<10 mg kg^{-1}) but willow (*Salix* sp.) grown on a contaminated soil with 69 mg water–soluble F kg^{-1} accumulated 18 mg kg^{-1} in the leaves. Plants on a control soil had only 1.5 mg kg^{-1}. This suggests a small amount of uptake but it also indicates a difference between species, a complication that has not been considered to any extent. Unfortunately, the report does not say whether the soil profiles were intact or whether plants were grown on material from only one horizon, but if whole profiles were used that might explain the differences between species provided they had different rooting behavior. In contrast, Sidhu [29] and Horntvedt [30] found no evidence of significant uptake from contaminated soil. Table 3 shows that at the site that Sidhu [22] investigated, the total fluoride in the humus and in what he called the ''available'' (i.e., TISAB buffer soluble) fraction were significantly higher than background. In 1975 the factory ceased emissions between

Table 4. The fluoride content (mg F kg^{-1} dry wt) in leaves of balsam fir (*Abies balsamea*) and black spruce (*Picea mariana*) growing in the vicinity of a phosporus factory in 1974 and 1975 (data from Sidhu [29])

	Zone I		Zone II		Zone III		Zone IV		Outside Zone IV	
Species	1974	1975	1974	1975	1974	1975	1974	1975	1974	1975
Balsam fir	251	24.7	54.1	7.6	19.3	7.4	10.4	3.7	7.2	5.2
Black spruce	370	8.1	34.3	8.7	14.3	6.5	7.1	3.9	5.2	3.7

May and October so Sidhu examined leaf injury, growth, and the fluoride content of the leaves of four species. There was no visible injury that year and there was an increase in growth compared with the previous year and, most importantly, foliar fluoride concentrations fell dramatically to background levels (Table 4). Sidhu [29] concluded that the very low leaf concentrations in 1975 indicated little translocation from the soil or from older to younger foliage. Using a different approach, Horntvedt [30] analyzed the relationship between emission rates from an aluminum smelter and the fluoride content of pine (*Pinus sylvestris*) needles growing in the vicinity, between 1967 and 1992. The smelter started operation in 1954 and there was concern about the possibility of long-term effects due to build up of fluoride in the soil [25]. The emissions varied from <20 to about 60 kg F h^{-1} over the 25-year period between 1967 and 1992. Needle fluoride concentrations ranged from about 30 to 60 mg kg^{-1} and correlated very well with the emission rates. Because of this, Horntvedt [30] concluded that there were no apparent long-term effects on needle fluoride from fluoride in the soil. As the soils were acidic in these three cases, the data appear to contradict the predictions made by Stevens *et al*. [14] from their model (Fig. 1). However, the discrepancy may be explained if the fluoride in the organic layers of both Sidhu and Horntvedt's soils was very mobile and leached out readily when atmospheric concentrations changed. Another possibility is that the trees had most of their roots in the mineral horizons where fluoride concentrations were not so elevated. Future investigations would benefit from an examination of rooting depths, the mobility of fluoride in the organic horizons, and the fluoride profile with depth.

3. IS FLUORIDE LOST FROM PLANTS?

The fluoride content of plants is very important for providing a basis for monitoring emissions and for preventing harmful effects on crops and fluorosis in grazing animals. In many countries, the amount of fluoride in vegetation is used as a regulatory tool to try to prevent these deleterious environmental effects. Although not as common, there are some jurisdictions that also regulate the amount of

fluoride in native vegetation and ornamental plants with the purpose of preventing damage to those species. There are probably tens of thousands of plant fluoride concentrations published in the literature and several useful studies of fluoride uptake, but given the importance of understanding the dynamics of plant fluoride, there are some surprising gaps in our knowledge. For example, it is a common observation that two species growing side-by-side may have very different fluoride concentrations in their leaves. This disparity may be due to differences in the rate of uptake, leaf age, or leaf area/weight ratio [1]. Rates of deposition (surface deposition plus uptake) can be estimated using standard methods, so if the concentration of fluoride in the air is known, and there is information about stomatal conductance and specific leaf weight, it is possible to predict the total fluoride uptake over short periods of time with a reasonable degree of accuracy. But there are some still unknown factors that make longer-term estimates less reliable or impossible and there have been few attempts to produce a model that can be used to predict the fluoride content of leaves of different species under a range of conditions. An examination and comparison of some data on changes in fluoride content of leaves over time will highlight one of the main problems that arises in any attempt to predict the fluoride content of leaves; fluoride is not only taken up by leaves from the air but also, the concentration may fall, often over short periods of time. The mechanisms underlying rapid decreases in concentrations are still not clear, more than 20 years after the problem was first raised [31]. Figs. 2–4 show data for changes over time in willow (*Salix caprea*), rowan

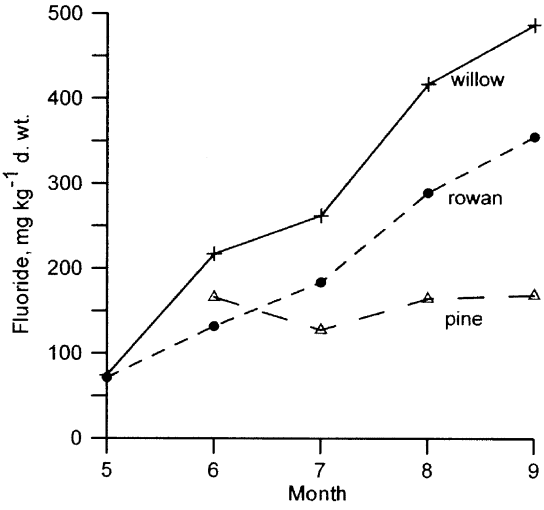

Fig. 2. The change in fluoride content in willow (*Salix caprea*), rowan (*Sorbus aucuparia*), and pine (*Pinus sylvestris*) growing close to each other in the vicinity of a Norwegian aluminum smelter between May and September. Re-drawn from Vike and Håbjørg [32].

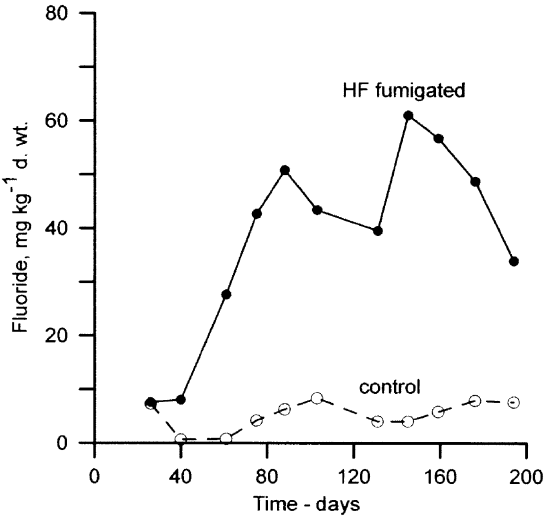

Fig. 3. The change in fluoride content in Shiraz grape leaves exposed in open-top chambers to a continuous mean concentration of 0.27 $\mu g\, m^{-3}$ F. Re-drawn from Murray [33].

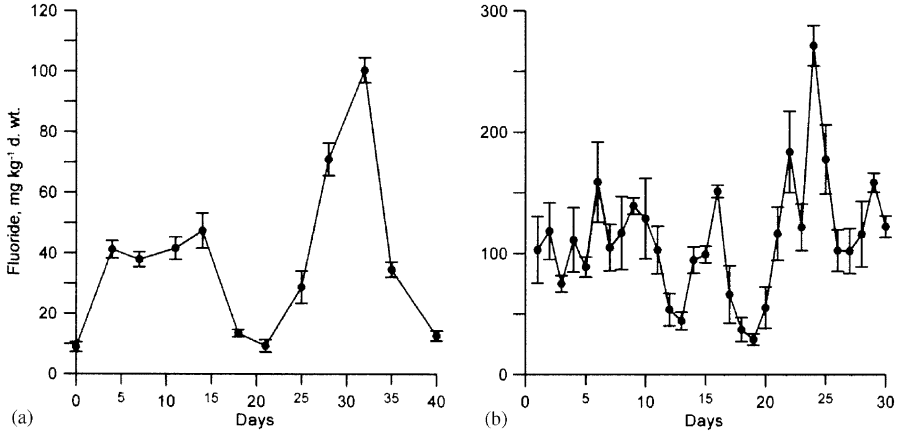

Fig. 4. Variation in the mean fluoride content (mg F kg^{-1} and standard error) of pasture grass at two locations: a) near a brickworks in the Netherlands (van der Eerden, 1981) and b) near an aluminium smelter in England (Blakemore, 1978). Both data sets were collected in the autumn when there was no growth. Re-drawn from Blakemore [34] and van der Eerden [35].

(*Sorbus aucuparia*), pine (*Pinus sylvestris*) [32], grape (*Vitis vinifera*) [33], and grass swards [34,35]. All of the work was in the field ([33] was in open-top chambers) in a range of climates.

Fig. 2 shows the change in fluoride content of three tree species growing close to each other in the vicinity of a Norwegian aluminum smelter between May and September. Although there undoubtedly were changes in the fluoride concentration in the air, the mean concentration in the leaves of two species showed a reasonably steady increase in fluoride over time. The second-year pine needles showed little change, probably because stomatal conductance was very low and there may have even been some occlusion of the stomatal pores by waxes or dust, limiting further uptake. The increase in the two deciduous species is typical of trees and it gives the impression of steady accumulation of fluoride over the season. There is no indication of any decrease or loss from the leaves. Murray's [33] data (Fig. 3) are for Shiraz grape exposed in open-top chambers to a continuous mean concentration of 0.27 $\mu g\, m^{-3}$ F (standard deviation 0.13). This is a low concentration but there was still significant accumulation in the leaves as the season progressed. However, after about 60–70 days there was an apparent fall, followed by an increase and a second fall. Some of the decrease may have been just sampling errors but it appears that grape is different in its fluoride dynamics from birch and rowan. Grass fluoride concentrations are typically even more dynamic, at least in the northern temperate parts of Europe where they have been most studied. Fig. 4 shows data on the fluoride content of grass swards growing near fluoride sources. In both cases, there were statistically significant falls in fluoride content over periods ranging from 1 to 10 days. Data published by Davison *et al.* [36] showed a loss of fluoride in a grass sward from 508 to 188 $mg\, kg^{-1}$ in a week. Why did these trees, grape, and grasses behave so differently and why did the concentrations decrease so rapidly?

A summary of published reports of rapid decreases in fluoride concentrations is given in Table 5. Note that some of the examples occurred after plants were exposed to HF and then placed in a clean atmosphere; the others occurred while the plants were still exposed to HF in the field. Several possible mechanisms were discussed by Davison [31] and Weinstein and Davison [1]. These were as follows: growth dilution; leaching by rain; leaf death; shedding of surface waxes; guttation; translocation to the stem and root; and volatilization.

The idea of growth dilution, the plant producing dry matter faster than it takes up fluoride, has been around for decades but Davison and Weinstein [1] concluded that it is only likely to happen when the rate of uptake is very low and the growth rate is very high. Also, it cannot account for many of the reported decreases (Table 5), because they occurred in tissues or situations where there was no growth, such as mature spruce needles and grass swards in the late autumn.

Table 5. Reported examples of rapid decreases in the fluoride content of plants (after Davison [31], with additions)

Author	Observation
Zimmerman and Hitchcock [37]	First report in literature in English. No amount of loss given.
Weinstein [38]	Large losses of F from bean and tomato leaves 3, 6, and 9 days after cessation of exposure.
Hitchcock *et al.* [39]	Up to 46–70% loss from maize leaves one week or more after exposure
Guderian *et al*. [40]	F decreased from 310 to 105 $\mu g\, g^{-1}$ in old rape plants, and from 465 to 62 $\mu g\, g^{-1}$ in young rape plants in 11 days.
Knabe [41]	Loss of up to 340 $\mu g\, g^{-1}$ from spruce needles; up to 200 $\mu g\, g^{-1}$ in washed needles after 5 months post fumigation.
Hitchcock *et al.* [42]	Loss of 50% in alfalfa after 8–22 days postfumigation.
Georgsson and Petersson [43]	Grass contaminated with volcanic ash fell from 4300 to 30 $\mu g\, g^{-1}$ after 30 days.
Davison and Blakemore [44]	Loss of up to 27 $\mu g\, g^{-1}$ from washed grass in 1 day, in the field, continuous exposure.
Ghiasseddin *et al.* [45]	Decrease of up to 180 $\mu g\, g^{-1}$ in 7 days.
Davison *et al.* [36]	Many examples of decreases in forage of over 50 $\mu g\, g^{-1}$ in 1 day in the field with continuous exposure.
Bunce (personal communication)	Loss of 40 $\mu g\, g^{-1}$ in 7 days in hemlock.
Van der Eerden [35]	Losses of up to 70 $\mu g\, g^{-1}$ in 10 days in grass in the field with continuous exposure.

Intuitively, the idea that rain leaches fluoride from leaf surfaces or the interior seems feasible and there are several reports of inverse correlations between leaf fluoride and rainfall that appear to support this concept [summarized in Ref. 1]. However, the situation is complex because rain scrubs the atmosphere, reducing the fluoride concentration (which reduces uptake), but it increases the dissolved or suspended fluoride that can be deposited on the surfaces. Furthermore, the rate of deposition of fluoride on a wet leaf surface is greater than on a dry one so there are conflicting forces at work. The inverse correlation between rain volume and plant fluoride content may be simply due to the lower fluoride content in the atmosphere. Furthermore, experimental application of rain does not support the

idea of leaching and in contrast, Less *et al.* [46] found that artificial precipitation resulted in an increase in grass fluoride concentration of about twofold. The answer to this puzzle may be that the effect of precipitation may depend on how heavy and frequent the showers are. Less *et al.* [46] concluded that, ''frequent rather than heavy rainfall would lead to greater absorption.'' The problem of the effects of rain remains unresolved.

Leaf death, shedding of surface waxes, and translocation to the stem and root were all considered to be minor or insignificant sources of change by Weinstein and Davison [1] because of the magnitude and speed of the reported changes. Grass leaves die and cuticular waxes are shed but not at such a speed as to cause changes within a day. Transport of fluoride from leaves to stems and roots (and perhaps loss from roots to the surrounding soil) is also unlikely because most of the evidence indicates that fluoride absorbed from the atmosphere is translocated to the leaf tips and not to any degree downward to stems or roots. For example, Ledbetter *et al*. [47] exposed tomato plants to ^{18}F and showed that the accumulation of fluoride was mainly in the leaves and glandular hairs of the petioles and stem, with little movement to the roots. But, Kronberger and Halbwachs [48] and Kronberger *et al*. [49] reported downward movement of fluoride in conductive tissues of spruce and accumulation in bark. However, some of the experimental data are equivocal [50] so this mechanism is not considered to be significant. Guttation is the expression of water droplets from the margins of leaves that occurs under certain conditions of temperature and humidity. It is common in grasses, and fluoride is detectable in guttation droplets, but Takmaz-Nisancioglu [51] thought that guttation could not account for the observed rates of decrease in grass swards. That leaves only one remaining mechanism, volatilization.

Volatilization has been invoked as an explanation of losses by plants, and it can be easily demonstrated, in principle, using artificial surfaces. For example, Takmaz-Nisancioglu [51] made $4\,cm \times 4\,cm$ filter paper squares impregnated with citrate-phosphate buffers to control pH over the range from 2.2 to 7.0. A sodium fluoride solution was then applied to the papers to give $60\,\mu g$ F per paper ($= 32\,cm^2$, both surfaces). Papers were hung in fluoride-free air, sampled at intervals, and analyzed. Fig. 5 shows the rate of loss of F in $\mu g\ m^{-2}\ day^{-1}$ plotted against the pH of the paper. The shape of the curve reflects the fact that HF predominates over F^- with decreasing pH and that HF is volatile. A grass leaf weighs about $20\,g\,m^{-2}$ so if the rate of loss is divided by 20 it gives an idea of the potential rate of loss from a leaf in $\mu g\ g^{-1}\ day^{-1}$. This is shown on the right-hand *y*-axis of the graph and it can be seen that under the conditions in the experiment, at pH values between 5 and 6, loss would be around $10–30\,\mu g\ g^{-1}$. Wind speed, the initial concentration on the paper, the shape of the model, and temperature all affected the rate of loss, but the experiment demonstrates that at moderately acidic pH, the rate of loss can be similar to that observed in the field

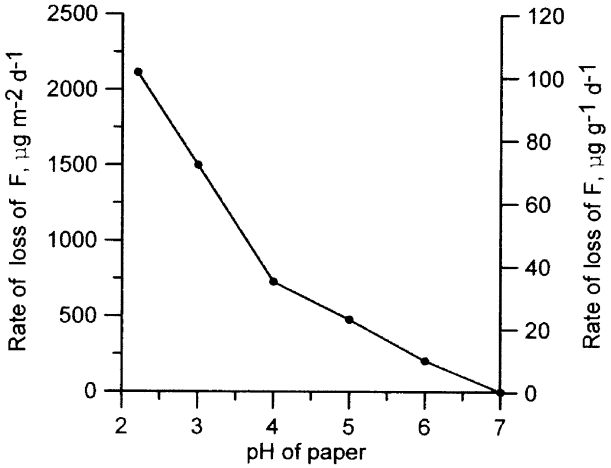

Fig. 5. The rate of loss of fluoride ($\mu g^{-2}\,day^{-1}$) plotted against the pH of 4 cm $\times$ 4 cm filter paper squares suspended in a well-ventilated space. The graph also shows the hypothetical rate of loss ($\mu g\,g^{-1}\,day^{-1}$) from a grass leaf. Redrawn from Takmaz-Nisancioglu [51].

for grass swards. The rate would be substantially less for thicker or more sclerified leaves so if this mechanism occurs it is probably of significance only for grasses.

If volatilization from leaf surfaces can occur, then theoretically it can also occur from inside leaves via the stomatal pores, provided the pH of the fluid in the cell walls is low enough ($<$ pH 6) to form HF and there is a difference in partial pressure between the inside and outside of the leaf. Surprisingly, there is experimental evidence that this can occur. Garrec and Chopin [52] attempted to confirm volatilization loss by analyzing the air blown over small fir (*Abies*) trees that were fed ammonium fluoride through the roots. They passed clean air over the trees, then filtered and analyzed it at the exit from the chamber. The rate of loss was measurable but low, which seems to confirm the possibility of volatilization from the interior of leaves. The low rate of loss may have been partly caused by the slow airflow in the exposure system and the low stomatal conductance of fir. Whatever the explanation is, this intriguing experiment needs to be repeated as part of a concerted study of the mechanisms by which the fluoride content of leaves can decrease so rapidly.

4. PROBLEMS CLASSIFYING PLANT SPECIES SENSITIVITY

The sensitivity or tolerance of plants to fluoride is almost universally based on the tendency of species to produce visible symptoms of injury. Once inside a leaf,

fluoride ions are carried with the transpiration stream of water from the vein ends through cell walls to the margins and tip, this causes accumulation of fluoride in the margins and this concentration mechanism is one of the main reasons for its extreme toxicity. If the concentration is high enough locally, cell membranes are damaged, the contents leak, and cells die. When the area dries, the necrotic patches turn tan, brown, or black, depending on the species (Plate 1, upper left) The dead tissue may drop off, leaving a distorted shape. At lower concentrations the fluoride may disrupt the extension of cell walls, more so at the margins, so the leaf becomes cupped or buckled (Plate 1, upper right). If sufficient fluoride diffuses into the chloroplasts it is thought to complex with magnesium and disrupt the chlorophyll molecules. This leads to chlorotic (yellow) areas between the veins (Plate 1, lower left). Species differ in their sensitivity by at least 2 orders of

Plate 1. (*upper left*). Necrosis of the tips of the current year's needles of lodgepole pine (*Pinus contorta*) (photo LHW); (*upper right*). Tip necrosis, distortion, and interveinal chlorosis in willow (*Salix* sp.) (photo AWD); (*lower left*). Interveinal chlorosis viewed by transmitted light in *Eucalyptus grandis*. There are also faint spots due to red pigmentation (photo LHW); (*lower right*). Suture red spot in Elberta peach. Note the reddening and soft tissue in the region of the suture (photo LHW).

magnitude, the most sensitive being visibly injured by exposure to ca. 0.3 $\mu g\, m^{-3}$ for prolonged periods.

Visible symptoms can be recognized by trained observers, but using such symptoms for classifying plants into categories of sensitivity or tolerance is fraught with many problems. One of these is that a species in one location may be sensitive while in another location, it may appear to be more tolerant. One example is *Eucalyptus globulus*, a species that is classed as of intermediate sensitivity in its native Australia [53], while in western Europe, where it is grown for pulp and timber, it appears to be more sensitive. This, of course, can be due to many factors, including the greater amount of precipitation in Europe, resulting in higher stomatal conductance [1]; but not to be ignored are differences in soil types, nutrition, and the genetic makeup of the population. Very little is known about the relative importance of these factors.

Over the years, many authors have published classifications of plants using the occurrence of visible symptoms near fluoride sources or occasionally in controlled experiments. The various classifications were discussed by Weinstein and Davison [1,54], but comprehensive lists have been offered by Weinstein [12], Guderian *et al.* [40], Doley [53], Borsdorf [55], Bossavy [56], Bolay and Bovay [57], Treshow and Pack [58], Dässler [59], Weinstein *et al.* [60], Weinstein and Hansen [61], and others. Although widely used, they are of limited accuracy for several reasons. First, a comparison of rankings is difficult because there are a limited number of species common to most lists. Second, sensitivity classes are subjective to the author and based on different criteria. Borsdorf [55] provides a good example. He divided species into four classes: *hochemfindlich* (highly sensitive), *empfindlich* (sensitive), *wenig empfindlich* (slightly sensitive), and *unempfindlich* (nonsensitive). The degree of sensitivity was based upon the degree of injury to a given species according to the distance from a single fluoride source in Central Germany. Thus, species that showed injury at the farthest distance from the source were the most sensitive, while those that exhibited little or no injury close to the source were tolerant. One assumes that the two other classes were intermediate in distance from the source but they were not defined by the author. An even simpler system was used by Bossavy [56] who divided species into only two classes: *sensibles* (sensitive) and *résistantes* (resistant). This was based upon observations in the French Alps in June, over several years, of the presence or absence of injury. The lack of consistency between the methods resulted in two different classifications of sensitivity for German iris (*Iris germanica)* by Borsdorf (*hochemfindlich*) and Bossavy (*sensibles*). Even more misleading is the classification of hawthorn (*Crataegus monogyna*) by Borsdorf as *wenig empfindlich* and by Bossavy as *résistante*. We have found that in the field all *Crataegus* species appear to be tolerant, unless exposed to a very high concentration of gaseous fluoride. Guderian *et al*. [40] used three categories to

describe injury: *sehr empfindlich (*very sensitive), *emfindlich* (sensitive), and *wenger empfindlich* (less sensitive). One might interpret the lack of a resistant category as meaning that all species exhibit some sensitivity to levels of fluoride found in the ambient air, but this is true only where there are unusually high concentrations or where high exposures are frequent. However, Borsdorf [55] also stated that many species are not injured even at the highest ambient concentrations. We have found that a very tolerant species such as *Juniperus virginiana may* gradually deteriorate after long-term relatively high concentrations. Even species that seem to be ''untouchable'' in most situations, such as camellia (*Camellia japonica*), rubber plant (*Ficus elastica*), privet (*Ligustrum ovalifolium*), or oleander (*Nerium oleander*), have been injured in areas immediately downwind of sources of high concentrations of HF. To add to this disorder, some authors have classified narrow groups of species, such as legumes, grasses, or grains according to their relative susceptibilities [40]. What these authors identify as the most susceptible species may be more commonly classified as moderately tolerant or tolerant on broader lists. So these different means of identifying the degree of a plant's susceptibility or tolerance have resulted in what appear to be anomalies in the classifications of some species.

An examination of the lists cited above reveals that many were based on fumigations carried out in closed chambers, usually with little or no control over temperature, humidity, light intensity, and often with rudimentary controls over HF concentration. The extensive list published by Weinstein and Davison [1], however, depends to a large degree upon field observations near fluoride-emitting sources but, unfortunately, in most cases, there were no corresponding measurements of atmospheric fluoride concentrations to give greater credence to the classification.

How can a consistent system of sensitivity classification be constructed? Certainly one requirement would be to use a common, objective basis for the classification. Ideally, atmospheric fluoride concentration should be used as the baseline for comparison. The most sensitive category would include those species that respond to the lowest concentrations known to cause injury (ca. 0.2–0.3 $\mu g\, m^{-3}$), while the most resistant would be those that tolerate the highest concentrations that commonly occur. If the range of concentrations is too narrow, however, this approach can be misleading. For example, Guderian *et al.* [40] exposed a number of plant species in Mylar greenhouses to 0.85 μg HF m^{-3} for 16 days or to 1.1 μg HF m^{-3} for 49 days, concentrations that are 3–4 times higher than the minimum concentration required to induce injury in some very sensitive species. In their study, both exposures resulted in ''very slight chlorosis'' in white clover (*Trifolium repens*), yet it was placed in the same sensitive class as *Gladiolus*, a classic bioindicator species that is injured at concentrations as low as 0.25–0.30 $\mu g\, m^{-3}$. In contrast, white clover was found to be insensitive by

Bossavy [56], a conclusion with which the present authors agree. There is little probability today that there will ever be sufficient funds, or adequate monitoring instrumentation, to conduct controlled studies where time, concentrations, effects of weather, nutrition, and response can be determined, even for a limited number of species.

Where does this leave us? Clearly, the numerous lists that have been published, notwithstanding the contradictions inherent between them, have value mostly as a aid to identification of symptoms observed in the field; if fluoride is the cause of symptoms then there should be much greater injury seen on sensitive species and less on more tolerant species. Furthermore, the degree of foliar injury can be used to map the ''footprint'' of emissions from a source, identifying areas where more investigation is needed. Annual surveys can be used to make comparisons from year to year in relation to changing emission rates or differences in the weather. Some plants are extremely valuable for diagnosing the presence of fluoride at concentrations that are so low that they are near the detection limits of automated measuring instruments. There are several species that have been used for mapping areas where fluoride injury occurs and they are termed as bioindicators. A bioindicator has been defined as ''a sensitive species that responds in a characteristic and predictable manner to the conditions that occur in a particular region or habitat'' [62]. This includes ''changes in leaves, flowers or fruits, and include formation of chlorotic (yellow) or necrotic lesions, other pigment changes, such as the production of red pigments (anthocyanosis), numerous kinds of leaf distortions, fruit deformities, reduced growth, alteration in plant form and other effects'' [1]. The most sensitive bioindicators are often termed as *sentinel* species, defined as ''the most sensitive organisms to fluoride in each plant or animal category.'' Some of the more useful species have been selected from the long list cited by Weinstein and Davison [1] and are shown in Table 6.

5. PROBLEMS ESTIMATING EFFECTS OF FLUORIDE ON PLANT GROWTH AND YIELD

Visible injury can be recognized in the field and it provides readily accessible evidence that fluoride concentrations have reached toxic levels but there is an obvious requirement to be able to assess the effects on the growth and yield of crops, trees, and other vegetation. Researching these effects is much more challenging than investigating visible injury so an obvious question is: whether there is a relationship between visible injury and effects on growth? If there is, it would assist assessment of the effects of fluoride sources. Logically, it would seem reasonable for there to be a relationship, especially if photosynthetic tissue is killed or leaves are lost. However, research on the growth of plants exposed to

Table 6. Relative sensitivities of selected higher plants to atmospheric fluorides classification on the basis of occurrence of foliar symptoms (data based on Weinstein and Davison [1])

Latin binomial	Common name	Response
Abies balsamea	Balsam fir	S
Acacia aulacocarpa, A. fimbriata, A. pulchella	Hickory wattle, Brisbane wattle, prickly moses	S
Acer negundo	Manitoba maple, box elder	S
Amelanchier canadensis	Service berry, Saskatoon berry	I
Avena sativa	Oat (young)	S
Avena sativa	Oat (mature)	T
Berberis vulgaris[a]	Common barberry	S
Betula lutea, B. nigra, B. papyrifera, B. pendula, B. populifolia	Yellow, black, white, European and gray birch	T
Cercis canadensis	Red bud	S
Chamaecyparis spp.	False cypress	T
Convallaria majalis	Lily of the valley	S
Cornus canadensis, C. florida	Bunchberry and flowering dogwood	T
Corymbia citriodora, C. intermedia	Lemon-scented gum, pink bloodwood	S
Epilobium angustifolium	Fireweed	I
Eucalyptus globulus, E. grandis, E. punctata, C. nudis, C. saligna	Tasmania blue gum, flooded gum, gray gum, western Australia flooded gum, Sydney bluegum	S
Ficus benjamina, F. elastica	Weeping fig, rubber plant	T
Fraxinus americana	White ash	S
Hosta spp.	Plantain lily	S
Gladiolus hortus[a]	Gladiolus, sword lily	S
Hypericum perforatum[a]	St John's wort, goatweed	S
Juglans cinerea, J. nigra, J. regia	Butternut, black, English walnut	I
Juniperus spp.	Juniper	T
Larix occidentalis	Western larch	S
Liquidambar styraciflua	Sweet gum	I
Maianthemum canadense, M. bifolium	False lily of-the-valley	S
Malus domestica	Apple	T

Table 6. Continued

Latin binomial	Common name	Response
Medicago sativa	Lucerne, alfalfa	T
Nerium oleander	Oleander	T
Picea glauca, P. pungens	White, Colorado spruce	I
Pinus strobus[a], *P. mugo, P. contorta, P. taeda, P. ponderosa, P. sylvestris*	WhIte, mugo, lodgepole, loblolly,ponderosa, scotch pine (young needles)	S
Platanus acerifolia, P. occidentalis	London plane tree, sycamore	I
Populus deltoides	Cottonwood	I–T
Populus tremuloides	Trembling aspen	S
Prunus avium	Sweet cherry	I
Prunus persica	Peach (leaves)	I
Prunus persica	Peach (fruit)	S
Pseudotsuga menziesii	Douglas-fir	S
Quercus alba, Q. palustris, Q. velutina, Q. virginiana	White, pin, black, live oak	T
Robinia pseudoacacia	Black locust	T
Sorbus aucuparia	European mountain ash, rowan	I
Thuja occidentalis	Arborvitae	T
Tsuga canadensis	Canadian hemlock	T
Tulipa sp.	Tulip	S
Ulmus alata, U. americana, U. parvifolia, U. pumila	Winged, American, Chinese, Siberian elm	T
Vaccinium spp.	Blueberry	S
Vitis vinifera	European grape	S
Xanthorrhea spp.	Grass tree	S
Zea mays	Sweet corn (many cvs.), maize	S
Zea mays	Field corn, maize	I

[a] Commonly used bioindicators.

other air pollutants such as ozone, sulfur dioxide, and nitrogen oxides informs us that the relationship between foliar symptoms and yield is usually, at best, tenuous. One reason is that these pollutants are metabolized after contacting plant tissues, and sulfur and nitrogen are essential plant nutrients. Fluoride, however, is a special case; it is not an essential element and normally it occurs in plants in only trace amounts as a contaminant from the soil, water, or atmosphere so a better relationship might be expected. Unfortunately, this is not the case; the relationship with growth and yield has rarely been shown to be significant. This is

important because most, if not all, fluoride air quality standards are based on the occurrence of visible effects on plants, whether as foliar lesions or other markings. These markings are adequate for establishing that the plant aesthetic has been degraded in residential landscapes and parks, and they can be used to protect certain soft fruit, but they do not guarantee that there are no effects on growth or yield. Therefore, this section reviews what is known about the effects of fluorides on growth and yield, drawing attention to the significant findings, shortcomings, and needs.

The quality and usefulness of all air pollution studies depend on the techniques that are available. A few studies have used tree rings to study the effects of fluoride on growth in the natural environment but most research on this subject, over more than six decades, has used controlled environments. This began with the use of glasshouses and other enclosed chambers to produce controlled concentrations of hydrogen fluoride or particulate materials. Although these closed chambers produced some informative data (e.g. Refs. [63–65]), lighting was often poor, temperatures too high, and inadequate air movement reduced pollutant uptake very significantly [1]. Their shortcomings were recognized in the 1960s and that led to the introduction of open-top chambers in which ambient air is blown into large transparent cylinders placed over vegetation in the field [66,67]. Rates of air movement are high to keep the temperature near ambient and the open-top chamber was designed to allow ingress of rain and insects, etc. They were thought to produce conditions that are a much closer simulation of the field environment and, hence they were considered to provide reliable data on yield. They became the workhorse of most air pollution studies, and current air quality standards for several pollutants are based on research using them. However, eventually it was realized that they too have certain limitations because they increase air and soil temperatures, reduce humidity, and alter the light environment by small but important amounts. The increased turbulence inside chambers usually results in greater uptake and deposition of the pollutant making it more difficult to quantify the relationship between the exposure and yield. In the last decade or so, there has been more use made of exposure systems in which plants grown in the field are not enclosed at all; so-called free-air systems [68–71]. These allow plants ranging from annual crops to mature trees to be exposed under real-life field conditions for long periods and they are currently the essential technique in investigations of the effects of elevated CO_2 and ozone. The significance of these changes in technology is that few of the studies of fluoride using enclosed chambers can be relied upon to provide useful data on growth or yield, while work using open-top chambers needs to be interpreted with caution. Unfortunately there have been no reported studies of fluoride using free-air systems, which may be due to the need for, and lack of, a fast-response, real-time analyzer that will work at sub-$\mu g\,F\,m^{-3}$ concentrations.

Table 7. Yield of field-grown wheat after exposure to HF for 4 days at two stages of development (after MacLean and Schneider [72])

HF ($\mu g\,m^{-3}$ for 4 day)	Yield per plant (g)	Spikes per plant	Weight per spike (g)
		Exposed at boot stage	
0	1.29a	3.46b	0.364a
0.9	1.03b	3.45b	0.306b
2.9	1.21a	4.65a	0.254c
		Exposed at anthesis	
0	1.57a	3.55a	0.488a
0.9	1.07b	3.26ab	0.333b
2.9	0.93b	2.99b	0.326b

Note: Means within a column followed by the same letter were not significantly different ($p = 0.05$).

There have been relatively few open-top chamber studies that have investigated fluoride but they all used realistic concentrations of HF and they have produced valuable data. We will discuss four studies as examples. The first is a study by MacLean and Schneider [72] in which wheat plants were grown in the field under background conditions of fluoride. When the spikes first emerged (the "boot" stage), plots were enclosed in open-top chambers and provided with air filtered through calcium carbonate and charcoal, or to 0.9 and 2.9 μg F m^{-3} for 4 days. This experiment was repeated when another set of plants had reached anthesis (i.e. when the anthers are released). The short duration of the experiments reduced much of the effects induced by enclosure. Both exposure regimes resulted in significant effects (Table 7).

Grain yield was reduced in plants exposed at the boot stage by 0.9 $\mu g\,m^{-3}$ because the spikes were smaller. Oddly, the 2.9 $\mu g\,m^{-3}$ treatment did not affect yield because the smaller spikes were offset by a greater number of spikes per plant. At anthesis, the yield reduction was due to smaller and fewer spikes. This demonstrated that short-term exposures can have important effects on seed production and that the timing of flowering and fertilization is critical in determining effects on grain yield. Furthermore, there were no symptoms of injury on the wheat plants. Some of the effects on grain yield may have been due to direct effects of fluoride on the fertilization process, which we discuss in detail later. Also, the authors found that there was no movement of fluoride from the foliage to the grains, which is in agreement with field observations that grain fluoride is usually low. Studies with grapes in open-top chambers have also demonstrated

the lack of movement to the berry [73]. This is significant because, as emphasized by Weinstein and Davison [1], there is no risk to the consumer from ingesting grains or berries from fluoride-exposed plants.

The results of MacLean and Schneider [72] also demonstrate the lack of relationship between sensitivity assessments based on visible injury and yield loss. In most classifications, young wheat plants are listed as being sensitive and mature plants as tolerant. But MacLean and Schneider's [72] data, using low concentrations of HF for only 4 days would suggest that wheat in the boot and anthesis stages are sensitive. The authors recommended that plants be classified on different bases depending on their use – prevention of aesthetic loss or crop yield – is logical but, unfortunately, there are too few data that can be used to assemble such lists.

The second example is on the effects of long-duration exposure to HF by MacLean *et al.* [74]. They exposed bean (*Phaseolus vulgaris*) and tomato (*Solanum esculentum*) to 0.6 μg F m^{-3} for 43 and 95 days, respectively (Table 8). Bean, which is classed as tolerant in terms of visible injury, exhibited normal growth and no foliar lesions, but had much reduced fruit mass and numbers. Although open-top chambers tend to increase the effects of pollutants, after allowing for this, we consider that the concentration and duration were representative of the exposures that still occur near many HF sources. There was no effect on the growth or fruiting of tomato (results not shown here), which is generally classified as intermediate in tolerance so the authors proposed that the effects of fluoride on fruiting and foliar injury are each independent of the other.

The third example involved exposing wheat and sorghum plants to three successive 3-day exposures at 1.6 or 3.3 μg F m^{-3}, which provides eight permutations of the concentrations plus a control [75]. The authors found that the yield of

Table 8. Effect of continuous exposure to 0.6 μg HF m^{-3} on the yield of bean plants (*Phaseolus vulgaris* cv. "Tendergreen") (MacLean *et al.* [74])

	Control	HF fumigated
Dry weight tops (g)	80.9	81.8
Dry weight leaves (g)	41.5	44.3
Dry weight stems (g)	39.4	37.5
No. of pods harvested	88.4	77.3*
No. of marketable pods	70.4	56.5
No. of unmarketable pods	18	20.8
Fresh weight pods (g)	424	337**
Fresh weight marketable pods (g)	391	298**
Fresh weight unmarketable pods (g)	34.3	40.5

* Significantly different from control, $p = 0.05$.
** Significantly different from control, $p = 0.01$.

wheat was not related to the mean concentration over all exposure periods, but to a weighted contrast between the first and the subsequent periods; yield of one hybrid of sorghum was related to a weighted mean of the second and third exposures. Anthesis was again shown to be the most sensitive period with respect to effects on yield. The important conclusion was that exposures of the same duration that result in the same mean concentration do not necessarily result in equivalent effects. This raises many problems in the design of appropriate experiments; the number of combinations of treatments, concentrations, crops, and varieties is immense, too many to contemplate a comprehensive study. They also suggested two reasons for this. First, sensitivity of the plant may be partially determined by the effects of preceding exposures, as indicated by Zahn [76] for SO_2, in which he suggested that one exposure might desensitize the plant to a subsequent exposure or potentiate the effects of a preceding exposure. Whether this is true for fluoride is not known. Second, when exposures occur over an extended period of time, the sensitivity of the plant can be altered by changes in the environment and the phenological stage of development.

The final example involves the production of a disorder called ''suture red spot'' of peaches [77]. In this unusual disorder, one or both sides of the suture ripens prematurely, expressed by a red area that extends into the flesh, and is usually located at the stylar end of the fruit (Plate 1, lower right). In Elberta peach trees enclosed in open-top chambers, four overlapping exposure periods of 30

Table 9. Development of suture red spot in Elberta peach fruits exposed to overlapping 30-day exposures to 2 μg HF m^{-3}. The first indication of pit hardening was on the 15th of June. The percentages are based on 150 fruits per treatment (data from MacLean *et al.* [77])

	Fruits in each severity class (%)			Concentration of F in fruits at harvest maturity (mg kg^{-1})		
Dates of exposure	With SRS	Slight	Moderate	Severe	Exocarp	Mesocarp
15 June–15 July	6.1	6.1	0	0	6.7	<1.0
30 June–30 July	71.9	50.0	18.0	3.9	5.3	1.0
15 July–14 August	97.8	52.7	37.1	7.8	6.6	<1.0
30 July–29 August	90.3	22.1	40.3	27.3	17.9*	9.2*
Control	<1.0	<1.0	0	0	3.1**	1.2

* Significantly greater than all other values in the respective columns ($p = 0.05$).
** Significantly lower than all other values in the respective columns ($p = 0.05$).

days to 2 μg HF m^{-3} resulted in a high proportion of abnormal fruit. The induction of suture red spot was associated with the period after pit-hardening when up to 98% of the fruits were affected (Table 9). Continuous exposure for 105 days at a concentration as low as 0.3 μg HF m^{-3} resulted in 95% afflicted fruit. It is believed that the symptoms are caused by the fluoride complexing with calcium in the suture region and altering cellular development. This example illustrates that a very low concentration of fluoride, 0.3 μg HF m^{-3}, can have a serious effect on crop quality and therefore the marketable yield. Even if there was no reduction in yield, the red spot destroys the sale value of the crop. It is also worth noting that 0.3 μg HF m^{-3} is the concentration cited in several air quality standards designed to protect crops and other vegetation.

The analysis of tree rings to establish a historical record and quantify the effects of fluoride exposure has been used occasionally for more than 50 years. Collecting an adequate number of samples for tree ring analysis is time consuming and analysis of the data is challenging because of the natural variability, environmental differences with site and over time, and the co-occurrence of stresses other than fluoride. First-class statistical analysis is essential. During the so-called ''pine blight'' period near Spokane, USA, Lynch [78] showed by tree ring analysis that growth of ponderosa pine trees was depressed by fluoride emissions from a nearby aluminum smelter. Ring analysis was also used in determining the validity of claims of diminished forest tree growth in Mosjøen, Norway, between 1964 and 1973 [79]. Tree cores from 147 plots, a total of 1332 samples, mainly of Norway spruce (*Picea abies*) were analyzed independently by two statisticians. The cores covered 13 years before and 13 years after the smelter started operation giving a ''before'' and ''after'' comparison. There was broad agreement between the two statisticians and it was concluded that growth was reduced by about 20% up to about 4 km downwind but there was no effect beyond 8 km. However, the more interesting facts were that there was no clear functional relationship between foliar fluoride content and growth reduction, and that there was great variability between trees within plots and in adjacent plots. The presence of SO_2 was a complication but its influence on tree growth was not investigated. This illustrates that although tree ring analysis is potentially useful, it is important, if not essential, to have data on all of the potentially important environmental variables for each plot of trees. However, if there are several pollutants present and there is spatial variability in nutrients and other environmental stresses, it makes the statistical analysis more demanding.

Similar, but even more ambiguous results were obtained as a result of another study in Washington, USA [80]. In this case, there was a clear relationship between foliar leaf fluoride and reduction in ring growth of Douglas-fir (*Pseudotsuga menziesii*) but the authors drew attention to the presence of SO_2 as a complicating factor and suggested that fluoride and SO_2 interacted. They implied that this was the reason for large growth reductions in areas where the fluoride

Table 10. Estimated atmospheric fluoride concentrations and effects on western hemlock (*Tsuga heterophylla*) over two time periods (data from Bunce [82])

Period	Zone	Estimated HF ($\mu g\,m^{-3}$)	[F] in foliage ($mg\,kg^{-1}$)	Change in basal area (%)	Change in wood volume (m^3)
1954–1973	Inner	3.42	271	−28.1	−12,703
	Outer	2.05	163	−19	−41,098
	Surround	1.3	104	−2.2	−4.212
1974–1979	Inner	109	87	−45.3	−3087
	Outer	0.34	29	2.8	+1192
	Surround	0.25	22	13.6	+4616

content of needles was relatively low. Because the SO_2 concentration was never measured, it was impossible to distinguish between effects of the two pollutants alone or in combination [80].

One of the most remarkable fluoride problems occurred in Kitimat, British Columbia, Canada, between 1956 (when a new aluminum smelter opened) and 1979. As a result of fluoride emissions (or one or more of many other factors present), there was an enormous outbreak of destructive insects in the area from 1961 until about 1969. Bunce [81] showed that growth reductions were evident until 1973 (after factoring out insect effects). They were estimated in three zones (termed ''inner,'' ''outer,'' and ''surround'') as 28.1, 19.0, and 2.2%, respectively, and were associated with calculated atmospheric concentrations of 3.4, 2.1, and $1.3\,\mu g\,F\,m^{-3}$. Astonishingly, when measured over a later period of greatly reduced emissions, Bunce [82] found that in the ''outer'' and ''surround'' zones, where the concentration was calculated to be 0.34 and $0.26\,\mu g\,F\,m^{-3}$, growth appeared to be increased by 2.8 and 13.6%, respectively (Table 10). The apparent stimulation of growth by low concentrations of fluoride has been reported many times (see Ref. [12]) and this type of response from many toxic substances has been termed ''hormesis.'' However, the mechanism of this effect of fluoride is not known.

6. HOW SIGNIFICANT ARE POLLUTANT INTERACTIONS?

Perhaps the poorest understood area of air pollution, in general, is the joint action of two or more pollutants in combination, either sequentially or concurrently. Although the mechanisms of interaction at the metabolic level are not known, the mechanism by which one pollutant can alter the uptake of another pollutant, in the case of SO_2, NO_2, and O_3, has been attributed to effects on diffusive conductance

of leaves [83]. The highly phytotoxic HF probably falls in the same category. Much is known about the effects of the common pollutants on stomatal conductance [84], but the complication is that SO_2, NO_2, and O_3 may both increase and decrease conductance, depending on the environment and the pollutant concentrations. So, for example, increased fluoride uptake might occur when it is present with low SO_2 and decreased uptake in high SO_2. In the case of joint action with NO_2, there is evidence that stomatal closure in the presence of NO_2 is offset by the effects of HF, which results in a greater fluoride accumulation [85]. Ozone has been reported [84] to reduce and increase stomatal conductance under different circumstances, but in general, reduction is more frequently reported so it would be expected to reduce fluoride uptake during episodes. Such episodes usually occur during warm, dry weather so leaves are often also water stressed to some degree and that also decreases stomatal conductance.

Each plant species appears to have its own distinct spectrum of responses to air pollutants, and for unknown reasons, it is not common for a species to be equally sensitive to two different pollutants. Giant ragweed (*Ambrosia trifida*) and lucerne (or alfalfa – *Medicago sativa*), for example, are very sensitive to SO_2, but are relatively tolerant to HF, while *Gladiolus* and Italian prune (*Prunus domestica* var. *italica*) are very sensitive to HF and relatively tolerant to SO_2. The developing needles of many pines (e.g., *P. sylvestris*), however, are sensitive to both pollutants. For a response to be understood of course, it must be studied experimentally, and the paucity of research is mostly responsible for our poor knowledge. On the other hand, those involved in studying interactions soon realize that the subject is very complex, for a number of reasons. The first problem is deciding on what concentrations of each pollutant should be used and what is to be measured: foliar lesions, growth, yield, or reproduction? The decisions affect the feasibility, design, and cost of the experiment. Two of the most important limitations are the lack of data on co-occurring pollutants in industrial atmospheres and the complex combinations of exposures that can occur when two or more pollutants occur sequentially. However, the most important problem, and it applies to all air pollutants, is a logistical one of how to devise a system for investigating the myriad of combinations of pollutants for anything other than two pollutants. Even three pollutants given at a single concentration involves eight combinations. Add to that the need for a minimum of three replicates, the expense of long-term exposures, and the costs become astronomical. This is an important problem because it is rare for there to be only a single pollutant in most atmospheres. For example, in atmospheres near aluminum smelters, HF is accompanied by variable concentrations of SO_2, O_3, and NO_x not to mention cryolite (Na_3AlF_6), several other fluoride-containing particles and considerable amounts of chlorine, chlorides, and hydrocarbons. A final problem is that the response to two pollutants, say, HF and SO_2, is different according to species, and probably variety. It is impossible to test all the combinations so we do not

know what happens for even one species when two or three pollutants are applied sequentially or concurrently, with the concentrations varying with time and meteorology.

So what is known about this difficult subject? Although there have been relatively few published accounts of the joint action of HF and other gaseous air pollutants, most of them consist of qualitative observations where the authors examined symptom expression, effects on growth, reproduction, and relative susceptibility of trees, crops, or ornamentals [86]. Continuous quantitative monitoring of the atmosphere was generally not available. There have been only a few experimental investigations of the joint action of HF with other gases, but they comprise such a paltry catalog as to limit what characterizes these joint actions.

Some pollutants are accumulated and metabolically transformed by plants, such as sulfur (from SO_2) and nitrogen (from NO_x), but others are broken down on entry to the leaf, such as ozone. Fluoride and sulfur are accumulated by plants as a nonessential and an essential element, respectively, but both are potentially toxic. Therefore, the accumulation of either element in combination is of importance, not only to the plant, but also, in the case of fluoride, to herbivores. If the accumulation is greater than additive, it constitutes an extra hazard to the consumers of these plants and they are exposed to an additional risk. If the accumulation is less than additive there would, of course, be reduced risk. The most consistent interactive effect reported has been a reduction in fluoride concentration by the presence of SO_2 (Table 11). In *Medicago sativa* (Mandl *et al*., unpublished) the presence of SO_2 reduced the fluoride content, and the presence of HF reduced the sulfur content. In all cultivars of lucerne, the diffusive conductance to gas exchange was decreased by SO_2 as was the leaf temperature, which explains the interaction. Reduced fluoride accumulation in the presence of SO_2 has also been reported for citrus [87] and for maize by Mandl *et al*. [88]. Nitrogen dioxide, a relatively nonphytotoxic gas, was also found to reduce fluoride accumulation in maize when applied with $0.5\,\mu g\,HF\,m^{-3}$, but not with $1.5\,\mu g\,HF\,m^{-3}$ [85]. As O_3 commonly reduces gas exchange rates, a reduction in fluoride accumulation would be expected but it was unaffected in *Phleum pratense* and *Dactylis glomerata*, but was lower in perennial ryegrass (McCune, unpublished).

It is reasonable to expect that if a gaseous pollutant reduces the accumulation of fluoride, it should also reduce the incidence and severity of foliar lesions but there is little evidence to confirm this idea. In a controlled environment study, Mandl *et al*. [89] reported the occurrence of bifacial elliptical foliar lesions on maize in the presence of 400–790 $\mu g\,m^{-3}$ of SO_2, but when about $0.5\,\mu g\,m^{-3}$ of HF was included, the number of lesions increased severalfold. These lesions occurred on a cultivar of maize that is more sensitive to HF than to SO_2. When the experiments were repeated under field conditions [88], the same lesions

Table 11. Data on the joint action of pollutant gases on the fluoride content of plants. ↑, increase in concentration; ↓, decrease in concentration (modified from Weinstein and Davison [1])

Species	Effect	Authors [Ref.]
Citrus	↓ F accumulation	Matsushima & Brewer [87]
Sweet corn (*Zea mays*)	↓ F accumulation by ca. 30%	Mandl *et al.* [88]
	↓ F accumulation by ca. 40%	Mandl *et al.* [89]
Lucerne (*Medicago sativa*)	↓ F accumulation by ca. 30% ↓ S accumulation by ca. 18%	Brandt [90]
Millet (*Setaria italica*)	↓ F accumulation by ca. 20%	
Italian ryegrass (*Lolium multiflorum*)	↓ F accumulation	
Gladiolus	↓ F accumulation by ca. 34%	McCune [91]
Lolium	↓ F accumulation by ca. 24%	
Maize (*Zea mays*)	No effect	
Lucerne (*Medicago sativa*)	↓ F accumulation by ca. 24% ↓ S accumulation by ca. 18%	Mandl, cited by McCune [91]
Wheat (*Triticum aestivum*) Barley (*Hordeum vulgare*) *Soybean* (*Glycine max*) Maize (*Zea mays*)	No striking effect on F accumulation, but SO_2 offset some of the effects of HF	Murray and Wilson [92–94]; Davieson *et al.* [95]
Peanut (*Arachis hypogea*) Bean (*Phaseolus vulgaris*)	Concentrations of F low	
Eucalyptus spp (4)	↓ F accumulation in two species ↑ F accumulation in one species	
$HF + NO_2$		

Table 11. Continued

Species	Effect	Authors [Ref.]
Maize, sweet corn (*Zea mays*)	↓ F accumulation with low [HF], no effect at higher	Amundson *et al.* [96]
Timothy grass (*Phleum pratense*)	↑ F accumulation	
Orchard grass (*Dactylis glomerata*)	↑ F accumulation	
$HF + O_3$		
Timothy grass (*Phleum pratense*)	No effect	McCune (unpublished)
Orchard grass (*Dactylis glomerata*)	No effect	
Rye grass (*Lolium perenne*)	↓ F accumulation	
Radish (*Raphanus sativus*)	↑ F accumulation	
Maize, sweet corn (*Zea mays*)	↓ F accumulation	

were identified, but they were also observed on untreated plants. So, when a foliar symptom that occurs in an atmosphere containing $HF + SO_2$ can also occur from some unknown environmental variable, it raises the question if the joint action of the two pollutants is to sensitize the plant to a hitherto passive environmental factor. But in the experiments of Amundson *et al.* [96] using the same cultivar of maize, the elliptical lesions were not seen and typical fluoride-induced foliar injury was the result of exposure to 1.5 μg HF m^{-3}. In other species, the predominant foliar symptoms were identical to HF injury, not to the other associated gas.

The joint action of HF with other pollutants is largely limited to the results shown in Table 11 because there has been very little work on growth or yield. In the environmental chamber studies of Mandl *et al.* [89] using HF and SO_2, there were no interactive effects on fresh or dry weight yields of maize. In comparable field experiments, however, there was a significant decrease in fresh and dry weights of stalks, and the effects were no greater than additive [88]. Research on these joint actions was terminated in the early 1980s for lack of funding and it is unlikely that it will be re-started because of the decline in fluoride and sulfur emissions.

7. HOW IMPORTANT ARE EFFECTS OF HF ON FERTILIZATION AND SEED SET?

One of the intriguing areas of research that remains relatively unexamined concerns the effects of fluoride on the fertilization process. When pollen is deposited on a receptive stigma surface, it hydrates, germinates, and pollen tubes grow down the style to fertilize the ovules. The process of tube growth is complex [97] but a key feature is that it is dependent on calcium gradients in the stigma and pollen tubes, and this makes the process susceptible to interference by fluoride. The effects of HF on fertilization and seed set were first described by two groups: Pack and his co-workers in the 1960s and 1970s [98–102] and by Facteau [103,104]. After growing tomato plants at two levels of calcium (40 and 200 mg L^{-1}) and exposing them to 6.4 μg HF m^{-3} for 22 weeks, Pack [98] found that fruit size was related to both calcium and fluoride levels, the smallest fruit being in the low calcium + HF treatment, and that HF increased seedlessness. He concluded that calcium played an essential role in fertilization and that fluoride interfered with it. Pack [100] showed that exposure to HF as low as 0.55 μg m^{-3} caused a small increase in strawberry fruit deformation that was brought about by lack of development of seeds and associated receptacle tissue (the swollen, edible part of the fruit). This happened because normal receptacle growth takes place only if fertilization and normal embryo development occur. Work by Sulzbach and Pack [101] showed that fluoride affected pollen tube growth and fruit set in tomato, whereas Facteau, Wang, and Rowe [103] reported fluoride interference with pollen growth in cherry. Further confirmation of the effects of fluoride on pollen was produced by Facteau and Rowe's [104] work on Tilton apricot. Pollen germination in vitro was unaffected by sodium fluoride but in fumigated, pollinated flowers tube lengths and the percentage of styles in which the tubes reached the base were reduced by HF. The effect was greater for high concentrations given for short times than for the converse, lower concentrations for longer periods. They also commented that although Tilton apricot and Napoleon cherry were both classified as being of intermediate sensitivity on the basis of leaf injury, Tilton apricot is more sensitive in terms of pollen tube growth. A direct connection between HF, pollen tube growth, and calcium was made by Bonte *et al*. [105,106] when they examined the effects of HF on strawberry fruit development. Fruit deformity occurred much more frequently when carpels were exposed to HF than the pollen-producing anthers (Table 12). When carpels were exposed, 74% of the fruit were deformed but only 11% when the anthers were fumigated. They concluded that the stigmatic surface was altered by exposure to HF, affecting pollen tube growth and subsequent fertilization. Using an electron microprobe, they were able to show that there was a significant accumulation of fluoride on and just inside the stigmatic surface and that the fluoride disrupted the calcium gradient in the stigma and style [107].

Table 12. Effects of periods of fumigation with 5.4 μg HF m^{-3} on the % of malformed fruit and wt. per fruit of strawberry (after Bonte [106])

Combination of treatments	Period of treatment			Results	
	Before anthesis	Flowering/ fertilization	Maturation	Malformation (%)	Mean wt per fruit (g)
1	HF	HF	HF	57	3.47 a
2	HF	HF	CA	58	4.49 b
3	HF	CA	CA	1.3	5.58 c
4	CA	CA	CA	2.7	5.71 c
5	CA	CA	HF	5.4	5.56 c
6	CA	HF	HF	42	5.45 c

Note: Plants were fumigated at different times during development to give six combinations of treatment: 'HF' indicates stages when plants were fumigated and 'CA' indicates when they were in clean air. Means within a column followed by the same letter were not significantly different ($p = 0.05$).

Table 13. Differences in the fluoride content of leaves and fruit, and in the flowering, fruit set and seed production in blueberry growing in a transect from a phosphate fertilizer factory (data from Staniforth and Sidhu [108])

	Sites					
	A2	Td	A4	A5	A6	9B
Distance from factory (km)	1.4	4.6	5.8	8.6	10.3	18.7
Estimated mean [HF] in air (μg m^{-3})	11.38	5.9	2.3	1.9	0.9	0.1
[F] in leaves (mg kg^{-1})	216	92	57	43	30	9
[F] in fruit (mg kg^{-1})	51	22	26	10	3	2
Leaf injured (%)	22	22	7.5	0	0	0
Flowers per plant	12.6a	15.2ab	14.8ab	16.4b	15.1ab	17.2b
Fruit set (%)	11	16	45	74	83	73
Seeds/fruit	17.6a	18.3a	28.8b	29.4b	33.0bc	41.8c

Note: The atmospheric concentrations of HF were estimated from deposition on alkali plates. Means followed by a different letter were significantly different ($p = 0.05$).

This graphic use of a microprobe demonstrates the potential of the technique in fluoride research but unfortunately it has been little used.

There is no reason to suppose that crops such as strawberry are unique in their reaction to fluoride but there appears to be only one study of fruiting and fruit/seed set in wild plants. It concerned conifers, raspberry, and blueberry [108,109]. The authors recorded reproductive performance in a transect downwind of a

phosphate fertilizer factory that emitted fluorides. Within a few kilometers of the factory the conifers were so badly damaged that it was impossible to tell if the effects on cones and seeds of conifers were indirect and due to massive growth loss, or direct and due to interference with the fertilization process. However, the data were clearer for blueberry (Table 13). In this species, leaves were injured within about 6 km of the factory but it was not very severe at that distance. Leaf fluoride contents were elevated all the way along the transect up to 10 km but fruit concentrations were relatively low. This is normal and it happens because of limited uptake by the fruit and the very low surface-volume ratio of the fruit [1]. However, the interesting data are the number of flowers per plant, the percentage of fruit set and number of seeds per fruit. Although it is impossible to conclude with certainty that differences were due to an effect of HF on the fertilization process, the fact that the number of flowers per plant was very little affected but fruit set and seed numbers were, are consistent with the idea. They indicate that there should be more studies of this type. With the appropriate examination of pollen tube growth in the plants in the field, it should be possible to confirm effects and to estimate the critical exposure that affects fertilization. Biologists might ask if there are subtle effects of HF on the seed set of wild species that are being missed because they are not so obvious. Which species are most sensitive to disruption of pollen tube growth? Are wind-pollinated species sensitive because they have huge stigmatic surfaces that are fully exposed to the air? They would certainly be good candidates for future research. If there are effects of HF on pollen tube growth, we must consider whether there is a potential effect on natural selection. Pollen tubes growing down a stigma compete in the race to fertilize ovules; the fastest growing grain fertilizes the ovule, so its genetic material will be passed on to the next generation. So if pistils or pollen differ in their sensitivity to HF, this may present a rapid method for natural selection to occur. The intriguing question that this raises is the genetic makeup of the selected adult. What adult characters might be inherited with the DNA from a fluoride resistant pollen grain?

8. WHAT DO WE KNOW ABOUT FLUORIDE AND INSECTS?

Insects are vitally important components of ecosystems, pollinating flowers, acting as food for other animals and playing vital roles in decomposition and nutrient cycling. Many are serious pests, yet little is known concerning the effects of atmospheric fluoride on insects, and their interaction with plants. How does fluoride affect the susceptibility of plants to insects and what are the effects of fluoride on the plants and the population of insects that it supports? There are three kinds of evidence for effects on insects that we can examine: correlations and observations, partially controlled experiments, and controlled experiments.

Together they show that there are some important effects but they also reveal apparent conflicts and highlight the importance of the need for further research on bioavailability to, and mode of action of fluoride on, insects.

There have been numerous published observations of insect populations (and other invertebrates) in the vicinity of fluoride sources and, as one might expect, many of them reported increased fluoride concentrations at various trophic levels [1]. Many conclude that they have observed decreases while others have reported increases in insect populations in relation to fluoride. However, few have provided information on either the composition of the atmosphere or of the other environmental conditions under which the effects occurred. A weakness of all these field observations is that it is not possible to separate the effects of fluoride from all the other possible environmental factors that can affect insect populations. We consider that observations and correlations like these are best used as starting points for research so in this discussion we focus on the next step, semi-controlled and controlled experiments.

Davies *et al.* [110] and Port *et al.* [111] worked with pine sawfly (*Diprion pini*) larvae collected in the field and a laboratory strain of the large white butterfly, *Pieris brassicae* respectively. Pine sawfly larvae were collected from the vicinity of an aluminum smelter and then reared in the laboratory on pine needles. Davies *et al*. [110] found that although the fluoride content of the needles was up to 170 mg kg^{-1}, and whole larvae initially contained up to 219 mg kg^{-1}, pupae contained no detectable fluoride. Also, there was no relationship between the fluoride content of the diet and pupal weight. A mass balance showed that 46% of the fluoride in the larvae was in transit in the digestive system and 23% was surface deposit. The larvae of *P. brassicae* showed a similar low rate of fluoride retention and there was no effect of up to 500 mg F kg^{-1} on growth or development [111]. These data indicate that in these species fluoride had no detectable effect because retention was very low. This in turn was probably because the high pH of herbivorous insect digestive systems produces the anion F^-, which has minimal bioavailability.

In contrast, there are several semi-controlled and controlled studies that have shown significant effects of fluoride. One of the first attempts at a controlled experimental study was on the growth and fecundity of the Mexican bean beetle (*Epilachna varivestis*) feeding on HF-fumigated bean plants over several generations [112]. Three major components of the plant–insect system, growth, development, and reproduction were each affected detrimentally. Growth of larvae was reduced by about half in each of the five successive generations and for unknown reasons, adult males were affected less than adult females. Although leaves of bean plants were not washed before their introduction to the beetles, one can presume that most of the fluoride ingested was from internal not external fluoride. So severe effects were found on growth, development, and reproduction in each of the five generations.

Table 14. Pupal weight of *Trichoplusia ni* after feeding larvae on noncabbage and cabbage diets containing fluoride (modified from Hughes *et al.* [113])

		Mean pupal weight (mg)			
		Noncabbage diets		Cabbage diets	
Treatment	[F] in diet	Male	Female	Male	Female
Control	10	232.7	215.5	238.9	217.5
NaF	60	219.9	208.5	236.8	216.5
NaF	210	186.8	178.5	218.8	212.5

Experiments with cabbage looper (*Trichoplusia ni*) larvae demonstrated effects of fluoride but also resulted in unanswered questions [113]. Larvae were cultured on two kinds of medium. When the larvae were raised on an artificial diet with sodium fluoride added, the results were as expected; a reduction in growth, survival, and pupal weight. However, addition of cabbage leaves reduced toxicity (Table 14). Fluoride added as HF-fumigated cabbage had no effect, even when the concentration in the diet was as high as 438 mg F kg^{-1}. Both treatments reduced the concentration of fluoride retained by the insects which was probably due to fluoride complexing with components of the leaf tissue and reducing availability.

One of the most examined cases, using field observations, semi-controlled, and controlled experiments involves the silkworm, *Bombyx mori*. The toxicity of fluoride-contaminated mulberry leaves to silkworm larvae has been recognized for decades and fluoride pollution has been a challenge to commercial silk production in China, Japan, and India. Several field studies have shown correlations between fluoride and changes in silkworms [114–118] while experimental feeding studies indicate that silkworm larvae are extremely sensitive to fluoride. It alters feeding rates, softens the cuticle, and reduces growth rate or may even cause death (see Ref. [1] for details). Metabolic studies in general indicate that fluoride affects not only larval metabolism [119], but also the nutritional quality of the larval food, the mulberry leaf. Food conversion efficiency may also be affected [120]. Wang and Bian [121] found that the toxicity threshold concentration in mulberry leaves is about 30 mg kg^{-1} dry wt), and the lethal dose is between 120 and 200 mg kg^{-1}. No mortality occurred up to 30 mg kg^{-1}, but between 30 and 50 mg kg^{-1}, the mortality was more than 30%. These studies provide a convincing example of fluoride toxicity to insects.

Overall, research on the effects of fluoride on insects has thrown up evidence that some species may be affected but others not, and there are some puzzling, unexplained results. In some species such as pine sawfly absorption of fluoride appears to be minimal so that it is extremely tolerant, yet cabbage looper larvae show a difference between fluoride presented in an artificial diet and presented in

cabbage. Silkworm larvae are intolerant to leaf fluoride contents higher than 30 mg kg^{-1} so these differences need to be explained. For this it is essential to investigate the bioavailability of fluoride to different species and, in particular, the mechanisms underlying toxicity and tolerance. Some recent work in China offers a possible system for making progress in both of there fields. Chen *et al.* [122] and Xu *et al.* [123] have investigated the fluoride tolerance of silkworms and have reported differences in tolerance between strains. This is probably the first research to demonstrate that there are within-species differences in fluoride tolerance in insects. The phenomenon is well known for plants, but not for animals. The same authors have also identified a molecular marker of fluoride tolerance and plan to use it to assist breeding programs. This not only offers the possibility of producing fluoride tolerant strains but also demonstrates that molecular methods could provide excellent tools for investigating bioavailability and the toxic mechanisms of fluoride in insects.

9. THE INCREASING IMPORTANCE OF ORGANOFLUORINE COMPOUNDS TO ANIMALS

There are about 30 organofluorine compounds of natural origin [124] but over a million have been manufactured by chemists, and the number continues to rise [125,126]. Natural organofluorine compounds are the result of geological and biological processes while the production of the huge number of manufactured compounds is due to the fact that fluorination produces properties that are invaluable to medicine, agriculture, and industry. Although most natural organofluorides are restricted in their distribution, almost everyone has contact with a range of manufactured compounds. Some have appeared in remote regions of the biosphere such as Antarctica.

The first naturally occurring organofluoride to be identified was monofluoroacetate [127]. This was the result of many years of investigation into the toxic principle of poisonous African plants of the genus *Dichapetalum*. Later, other species were found to contain the same compound and a few were also found to contain ω-fluorinated fatty acids. All of these compounds are highly toxic to mammals but they have been reported as occurring in only a few genera in South Africa, Australia, and South America (Table 15). Nevertheless, their effects on livestock health and human social history have been profound [1]. In Australia, for example, monofluoroacetate played a major part in controlling European settlement of western regions and they continue to kill large numbers of sheep and cattle.

An obvious feature of the list in Table 15 is that the species are in several families that are separated both geographically and in the evolutionary sense. There are many species of *Gastrolobium* and a number of *Dichapetalum* species that produce organofluorides but *Acacia georginae* and *Palicourea marcgravii* are

Table 15. Species known to contain toxic organofluorides, mostly in the form of monofluoroacetate (data from Weinstein and Davison [1] with additions)

Genus/species	Family	Region/country
Gastrolobium, Oxylobium, Nemcia (the latter two genera are now included in *Gastrolobium*)	Fabaceae	Australia
Acacia georginae	Mimosaceae	Australia
Cyamopsis tetragonolobus	Fabaceae	India, Pakistan, grown as a crop in many countries
Dichapetalum	Dichapetalaceae	Tropics: Africa, Borneo, China, Philippines, Brazil, Mexico
Palicourea marcgravii	Rubiaceae	Brazil
Arrabidea bilibiata	Bignoniaceae	Brazil
Spondianthus preussii	Anacardiaceae	Cameroon, Niger, Ivory Coast

the only members of large genera that are known to produce these compounds. *Cyamopsis tetragonolobus* is unusual in that it is widely cultivated as a crop and used as cattle fodder. A product, guar gum, is used in dairy products such as ice cream and as a stabilizer in cheese and cold-meat processing. The fluoroacetate content [128] is reported as being very low (0.07–1.42 μg fluoroacetic acid g^{-1}), much lower than most of the species in Table 15 apart from *Palicourea marcgravii*. Vartiainen and Gynther [128] state that 'the low concentrations of fluoroacetate … dispel any considerations about possible health risks associated with fluoroacetate during the prolonged use of guar gum.'

The fact that unrelated species have the capacity to synthesize fluorinated organic compounds indicates that it has evolved independently several times. Research in Australia suggests that accumulation of fluoroacetate evolved as a defense mechanism against herbivores and that, in common with other plant defense systems, some animals have evolved tolerance [129–133]. The circumstances that lead to one species in a genus evolving the capacity to manufacture and tolerate monofluoroacetate while related species living in the same region do not, are not known. The fact that the capacity to synthesize organofluorides has evolved several times may also mean that there are differences in the metabolic pathways between species but the pathway or pathways are still not clearly defined. The problem in investigating the biochemistry of these compounds in

plants is that some of the species are difficult to cultivate, are slow growing, and the concentrations of organofluorides are very low. *Cyamopsis tetragonolobus* is a fast growing annual but the concentrations are very low, which may limit its use for investigations. Until recently the analytical detection and quantification of organofluorides was also difficult. Furthermore, the half-life of fluorine isotopes is too short to be of much use in tracing biochemical pathways.

Organofluorine accumulation by plants is known because of the toxicity of monofluoroacetate to livestock and humans so attention has focused on those species. However, it is possible that organofluorides also occur in other species, particularly those that are not eaten by livestock or, as in the case of *Cyamopsis tetragonolobus*, in concentrations that are too low to be toxic. In Australia, symptoms of toxicity are well known so it is unlikely that there are any new families or genera that wait to be discovered there. Similarly, in Africa *Dichapetalum* has been investigated but it is a large genus that is found across the warmer latitudes, including places as far apart as Borneo and Mexico. There are no reports of members of this genus being toxic outside of Africa (Prance personal communication) but there have been no systematic searches for the compounds in other countries. In South America, fluoroacetate has been identified in two unrelated species but again, there has not been a thorough search for others in the same genera or families. *Palicourea marcgravii*, the better known of the two, is a member of a large genus and some are used medicinally so there may be other species that also contain fluoroacetate. Bearing in mind the species richness of South America and tropical Asia, it would seem to be unlikely that there are not other species of organofluoride accumulators in those regions.

There are three known organofluorine compounds of microbial origin: the antibiotic nucleocidin (4′-fluoro-5′-*O*-sulfoamoyladenosine), 4-fluorothreonin, and monofluoroacetate. All were discovered accidentally during research on antibiotics. Nucleocidin was isolated in 1957 from *Streptomyces calvus* that was obtained from an Indian soil sample but it was not until much later that it was realized that it contained a fluorine atom. The metabolite has not been re-isolated from cultures so it is impossible to elucidate the fluorination mechanism [134]. This is unfortunate because O'Hagan and Harper [134] have suggested that, because of the location of the fluorine atom on the ribose moiety, the fluorination process in this organism may be different from that in other organisms. Clearly, efforts to reisolate the organism from soil are needed but it may never be available again for laboratory study [134]. The other two microbial compounds, 4-fluorothreonin and fluoroacetate, were discovered in *Streptomyces cattleya* [135]. This isolate is still available and it has provided a useful system for the investigation of organofluorine synthesis in microorganisms. However, in relation to our present concern, fluorides in the environment, it is not known if either *S. calvus* or *S. cattleya* produces organofluorine compounds in nature or if they have any environmental significance.

Organofluorides produced by geological processes are mostly of more recent discovery. They have been reported as occurring in minerals such as fluorite and in emissions from volcanic gas and thermal springs [124]. At present, over 20 have been identified, including a number of fluoroalkanes with climate forcing potential such as perfluoromethane, CF_4 (CFC-14). There are still questions about the quantities of some of the geological compounds that are emitted and analytical problems such as ways of extracting them without altering their chemical composition and preventing contamination of samples. However, readers are recommended to consult Gribble [124] who summarized the unresolved issues relating to these compounds, notably questions on the origin of the carbon and the mechanisms of their formation.

Manufactured organofluorides include pharmaceuticals, insecticides, pesticides, fluoropolymer fabrics, surfactants, refrigerants, aerosol propellants, non-stick surfaces for cookware, and chemical resistant tubing [1]. Some of these, such as inhalation anesthetics and insecticides are released directly into the atmosphere but many are solid and end up in land fill or incinerators. Agrochemicals in particular find their way into the soil and in some cases water bodies. For many years there was no great concern about the environmental fate or effects of manufactured organofluorides but the well-known case of chlorofluorocarbons (CFCs) changed that position. They had an enormously beneficial effect on the lives of millions of people but they were eventually shown to persist in the stratosphere, have serious effects on the ozone layer and to contribute to climate forcing (see Ref. [1] for a historical account). This highlighted the persistence of some organofluorides and demonstrated the need for greater vigilance and risk assessment in relation to the environment.

The chemical transformations and fate of manufactured organofluorides in the environment vary greatly but they can be considered as falling into two categories: (1) those that are readily defluorinated and biodegradable and (2) those that are resistant to defluorination and biodegradation. Based on research on monofluoroacetate and on the breakdown products of fluorinated inhalation anesthetics and hydrochlorofluorocarbons [136] the evidence suggests that mono- and difluoro methyl groups are readily defluorinated by biological means. For example, the old anesthetic methoxyflurane ($CHCl_2$–CF_2–O–CH_3) breaks down in the body to produce oxalic acid and inorganic fluoride, while extensive studies in New Zealand and Australia have shown that when monofluoroacetate is used as a rodenticide, it rapidly breaks down in soil [137–140]. This rapid defluorination means that in general compounds in which the fluorine is present in mono- and di-fluoro methyl groups do not accumulate and probably have little environmental impact. In contrast, many agrochemicals and pharmaceuticals contain tri-fluoro methyl groups while solids such as fabrics, insulation, tubing, and cookware linings are fluoropolymers. These are resistant to defluorination in the environment and have limited, if any, biodegradability. This was brought home by

research into the breakdown of hydrochlorofluorocarbons (HCFCs), the replacements for CFCs [136]. The HCFC risk assessment identified one of the important breakdown products as being trifluoroacetic acid (TFA). The risk assessment [128] also showed that although TFA has low animal and plant toxicity, it is not defluorinated in soils or water so it accumulates in the aquatic environment [141]. By the mid-1990s it was clear that TFA was detectable in the air, rain, fresh water, and oceans in most parts of the world. There are two other known sources of this compound, the breakdown of some inhalation anesthetics and the combustion of fluoropolymers but calculations showed that the concentrations and the total amount in the biosphere were far higher than could be accounted for by all the known industrial sources [136]. Frank *et al*. [141] concluded that the source of the massive reservoir of TFA in the oceans is ancient and that it pre-dates industry. They consider that the oceans are a final sink for a natural source whose nature is unknown and that the TFA in the other compartment is mostly of anthropogenic origin.

The research on HCFCs and TFA happened because of the effects of CFCs and the concern that they generated but there has not been comparable interest in the many thousands of other persistent organofluorides. The first indication that organofluorides can be transferred from the immediate area of their use started with a report by Taves [142] of the occurrence of an organofluorine component in human serum. The analytical procedure available at the time was not specific but the observation was later confirmed and in 1976 the use of NMR

Table 16. Examples of the concentrations of perfluoro-octane sulphonate (PFOS, $C_8F_{17}SO_3$) found in various animal tissues (data from Geisy and Kannan [144])

Group	Species	Location	Tissue	*N*	Range of concentrations ($ng\,g^{-1}$ wt tissue)
Birds	Polar skua	Antarctica	Plasma	2	<1–14
	Black-tailed gull	Hokkaido, Japan	Plasma	24	2–12
	Cormorant	Italy	Liver	12	33–740
	Bald eagle	Mid-west USA	Plasma	26	1–2570
Fish	Yellow-fin tuna	North Pacific	Liver	12	<7
	Blue-fin tuna	Mediterranean	Liver	8	21–87
	Chinook salmon	Michigan, USA	Liver	6	22–170

suggested that the compound might be perfluoro-octanoic acid, which is used in the manufacture of fluoropolymers [143]. More recent work using better analytical techniques has revealed that a number of perfluoro compounds occur in the serum of the general population and in animal tissues collected in regions that are remote from industry and human habitation (Table 16). This work, principally by Geisy and Kannan and colleagues [144–150] demonstrated that compounds that we might think of as being restricted to industrial and urban situations, do in fact find their way into most parts of the biosphere. The pathways and rates of movement are not known and much more information is needed on the animal toxicity. Geisy and Kannan [145] concluded that ''*knowledge of the critical mechanisms of toxic effects is needed to select appropriate endpoints and biomarkers of functional exposure and to assess complex PFC* (perfluoro chemical) *mixtures and their relationship to one another and to other environmental residues*.'' The same can be said for other groups of fluorinated compounds such as the residues from herbicides, pesticides, and pharmaceuticals so we finish by supporting Key, Howell, and Criddle [151] who concluded that ''*Research is needed to assess the fate and effects of non-volatile fluorinated organics, the fluorinated impurities in commercial formulations*….''

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