# THE METABOLISM OF AMMONIUM FLUORIDE AND SODIUM MONOFLUOROACETATE BY EXPERIMENTAL *ACACIA GEORGINAE*

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*ABSTRACT*

*Plants of* Acacia georginae (*one of numerous toxic tropical species now known to contain monofluoroacetate*) *were cultivated in nutrient-washed quartz, and in soil. Attempts were made to induce the formation of organic fluorine by treatment of the roots with a solution of ammonium fluoride. Only small amounts of carbon–fluorine material were measured in the leaves and roots, and examinations by physico-chemical methods failed to detect any evidence of the presence of monofluoroacetate in any of the plants. Similar plants were treated with sodium monofluoroacetate which underwent considerable degradation to an acid-labile form of fluorine* (*probably inorganic fluoride*). *The results of the analyses of the roots and leaves for fluorine revealed that the difference between acid-labile* (*diffusible*) *fluoride and total fluorine cannot be taken as a measure of the organic fluorine.*

## INTRODUCTION

Since Marais (1944) first reported the existence of monofluoroacetate in the leaves of the highly toxic South African plant, *Dichapetalum cymosum*, this and other naturally-occurring organic compounds of fluorine have been found in an increasing number and range of tropical species (De Oliveira, 1963; Ward *et al.*, 1964; Aplin, 1967; Vickery *et al.*, 1973).

In recent years several groups of workers have described experiments which it is claimed have demonstrated the synthesis of fluoroacetate and fluorocitrate by plant tissues. The Australian *Acacia georginae* (one of the toxic species) has been the plant most widely studied, but some common pasture and agricultural species have also been used (Peters *et al.*, 1965; Preuss *et al.*, 1970*a*, *b*; Peters & Shorthouse,



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1972*a*, *b*; Cheng *et al.*, 1968). Further, there have been reports by Lovelace *et al.* (1968), Cheng *et al.* (1968) and Yu & Miller (1970) that plants of ordinary pastures may contain fluoroacetate and fluorocitrate resulting from the metabolism of industrial effluents from hydrofluoric and phosphoric acid manufacture, which, in some circumstances, cause extensive environmental pollution. If such findings are confirmed an ecological situation of some gravity needs to be recognised since monofluoroacetate and related longer chain carbon–fluorine compounds are among the most toxic substances to be found in any plants and seriously affect the mammalian central nervous system and the heart, causing violent convulsions and ventricular fibrillation (cardiac arrest) (Peters *et al.*, 1953; Peters & Hall, 1960). It has already been shown that some of the tropical species which contain these extremely poisonous chemicals grow in soils that are not high in fluoro-minerals, indeed, some of the soils contain very little fluorine at all (Hall, 1972). These particular plants (which grow in Africa, Australia and Brazil) are therefore true accumulators of the element and nothing is known of the mode of synthesis of the organically bound forms of fluorine. Studies of the subject are fraught with technical difficulties, but the final proof of synthesis must be the isolation and determination of fluorine (sometimes in extremely small amounts) in the organic entity.

In the course of a systematic analytical examination of a wide range of these tropical plants, known to contain fluoroacetate and other longer chain fluoro acids, a means was devised to extract selectively the carbon–fluorine compounds so that inorganic fluoride did not interfere with, or confuse, the interpretation of the organic fluorine partition. Using this technique it was possible to assess the true organo-fluorine fraction in the various tissues (Hall, 1972; Hall & Cain, 1972). This present paper describes an attempt to induce the formation of organic fluorine in experimentally-cultivated *Acacia georginae* and with the selective extraction mentioned above to differentiate between inorganic and organic forms of fluorine, to measure the degradation of fluoroacetate by this plant. The results are discussed in relation to the possibility of such compounds being formed in plants which grow in fluoride-polluted environments.

## THE CULTURE OF EXPERIMENTAL *Acacia georginae*

The seeds of *Acacia georginae* were kindly supplied by the staff of the Experimental Station of the Commonwealth Scientific and Industrial Research Organisation at Alice Springs, Northern Territories, Australia. Many attempts were made to grow *Acacia georginae* under heated glasshouse and laboratory conditions in the North East of England before good plants were successfully raised.

The seeds were germinated on wet filter paper in a petri dish after sterilising the seedcoat by immersing in 1:1 ethanol/20 volume hydrogen peroxide solution for five minutes. The seeds were then rinsed, together with the filter paper and dish,

with sterile distilled water and left covered and shielded from bright daylight at a temperature of approximately 25°C. Within 48 h the seed coating had burst and a radical 1–2 cm long had emerged with the two well-formed cotyledons. At this stage the seedlings were placed into the selected growth medium. The germination rate was usually over 80% even with seeds several years old.

The two most successful methods for producing good plants were pot culture in a good quality loam soil or in white Norwegian quartz drenched with nutrient solution. In both systems 10 cm polypropylene pots were used with a layer of washed earthenware chips in the base. The soil was passed through a sieve of 5 mm mesh. The Norwegian quartz (obtained from the Compagnie Commerciale du Nord, 8, Queen's Walk, London NW9) was of 8–16 mesh size (1–2 mm). Before use, it was left for several hours in 2 M hydrochloric acid, then washed with water until free from acid and drenched with nutrient solution after filling the pots. The surface of the quartz at the top of the pot was covered with a disc of black polythene having a slit from the periphery to the centre so that it could easily be removed and replaced during treatment with the nutrient solution. The black disc prevented algal growth and minimised loss of moisture due to evaporation.

The nutrient solution was a modification of that described by Hewitt (1952) for general plant culture. The ferric citrate content was increased to 0·12 g/litre of solution to prevent leaf chlorosis which tended to develop without the use of the additional iron. In practice it was found convenient to make a solution five times the actual working strength and to store it in a polythene container at 2°C. The trace nutrients were added from a mixed concentrated solution (*i.e.* × 100). The concentrated ( × 5) nutrient solution was diluted with distilled water immediately before use, thus minimising temperature variations. Distilled water was used for the preparation of all solutions in these experiments. The working nutrient solution contained 1·04 $\mu g/ml^{-1}$ of fluoride and the quartz $< 1{\cdot}0$ $\mu g/g$.

## TREATMENT OF *Acacia georginae* WITH FLUORIDE AND FLUOROACETATE

The selected plants had been cultivated collectively in quartz for 14 months and were good healthy specimens, 20–35 cm high from the base of the stem to the top of the foliage. All had well developed, active root systems. They were re-planted singly in polypropylene pots (10 cm diameter) in newly-washed quartz (drenched with nutrient solution) or in an organic sandy clay loam and allowed to re-establish for four weeks. Four groups, each of four plants, were given the following treatments applied to the roots.

*QFa* 1–4 (*re-established in quartz*) were drenched every second day with the nutrient solution to which had been added 500 $\mu g/ml^{-1}$ of sodium monofluoroacetate (equivalent to 95 $\mu g/ml^{-1}$ of fluorine). Approximately 100 ml of the nutrient solution was used on each occasion and applied to the top of the quartz

from a polythene wash bottle, care being taken not to spray the foliage. The excess nutrient solution was removed from the saucers and the plants were left in a partially closed propagating cabinet at a mean temperature of 25°C.

*SFa* 1–4 (*re-established in soil*) were treated with an aqueous solution of 500 $\mu g/ml^{-1}$ of sodium monofluoroacetate once every five days with approximately 100 ml of solution. The soil was left moist but not waterlogged.

*QFd* 1–4 (*re-established in quartz*) were treated every second day with the nutrient solution (in the same manner as were the fluoroacetate-treated plants) containing 100 $\mu g/ml^{-1}$ of fluoride as the ammonium salt. To prevent precipitation of the fluoride from the nutrient solution, calcium nitrate and magnesium sulphate were replaced by ammonium nitrate and ammonium sulphate (0·066 g and 0·198 g/litre, respectively).

*SFd* 1–4 (*re-established in soil*) were treated once every five days with approximately 100 ml of ammonium fluoride solution containing 500 $\mu g/ml^{-1}$ of fluorine. The fluoride was increased to compensate for that which was expected to be immobilised by soil cations.

*Control plants* were grown in soil receiving only distilled water or in quartz drenched with nutrient solution which did not contain fluoride or fluoroacetate. These were not strictly 'controls' but merely served as 'normal' plants.

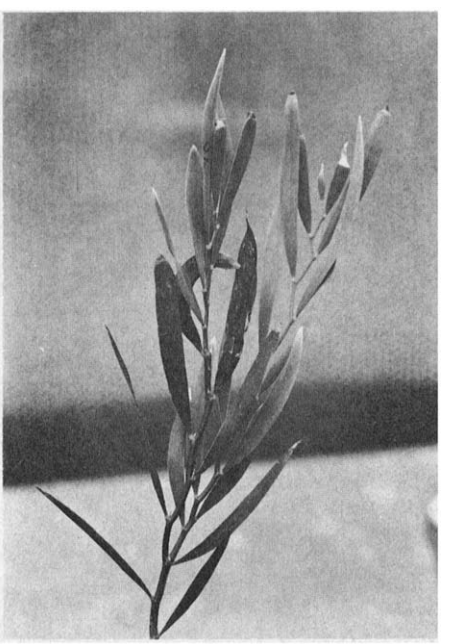

Fig. 1. *Acacia georginae* showing leaf-tip burn after treatment with ammonium fluoride.

*Growth and harvesting*

After two weeks the fluoride-treated plants exhibited leaf-tip burn characterised by desiccation of 0·5–1·0 cm of the tips of the top leaves. The leaf tips became slate blue in colour and curled (Fig. 1); visual damage to the plants was, however, slight. Treatment was discontinued after eight weeks.

*Preparation of plants for analysis*

All the leaves were removed with scissors and immediately weighed. The stems were then cut off at the soil or quartz surface and also weighed. The stump and roots of the plants grown in quartz were gently removed by placing the entire pot almost horizontally in a bowl of distilled water so that the quartz fell out. The roots were then very quickly immersed for a few seconds in three changes each of two litres of distilled water. Adhering quartz easily fell away, leaving the root systems remarkably intact. The roots were immediately blotted dry and quickly weighed. The roots of the fluoride-treated plants and one of the fluoroacetate-treated plants had clearly been adversely affected by the treatments (Fig. 2).

Fig. 2. Roots of experimental *Acacia georginae*. Top—Control plants (no treatment); Bottom—After treatment with ammonium fluoride.

The roots of the plants grown in soil were loosened by gently tapping the pot and removing the plant with the soil intact. All these plants had retained well formed root systems. The soil was carefully disturbed so that a high proportion fell away from the roots. Some root fragments were left in the soil but a visual inspection indicated that less than 5% of the whole root system was lost in this way. The roots were washed first with tap water, then with distilled water, in the same way as were the roots from the plants grown in quartz. Again the root systems remained intact and were virtually free from adhering soil.

The tissues were air-dried at 25–30°C for several days before being finely ground (50 $\mu$m particle size) using a Casella grain mill followed by ball milling (Hall, 1972). All the leaf and root tissues of the treated plants were analysed separately for organic fluorine, *i.e.* fluorine soluble in alkaline propan-1-ol, acid-labile, *i.e.* diffusible fluoride, and for total fluorine after fusion of the plant ash with potassium hydroxide (Hall, 1968; 1972). The leaf and root tissues of the control plants were pooled separately and similarly analysed.

The soil residues of the control plants and of fluoride-treated and fluoroacetate-treated plants were pooled separately and analysed for total fluorine, acid-labile fluoride, and water-soluble fluorine. Plant tissues and soils were also examined by infrared spectroscopy and subjected to electrodialysis (a technique for removing and concentrating small amounts of fluoroacetate from plant tissue and soil), the electrodialysates being analysed for total fluorine and inorganic fluoride, and for fluoroacetate by gas–liquid chromatography. The methods employed for all these procedures have been described elsewhere (Hall, 1972; Hall & Cain, 1972) except that in the analyses reported in this paper the organic fluorine was extracted into alkaline propan-1-ol containing molar lithium hydroxide instead of ammonium hydroxide.

## RESULTS

The relative weights of the roots to the aerial parts of the plants for both fresh and dry tissues are shown in Table 1 and it can be seen that the weights of the leaves and roots are approximately the same in most cases. Although the stem weights are not shown, they represented 6–12% of the plant; the roots alone were 30–50% of the whole plant.

*Partition of fluorine in dry tissues of experimental* Acacia georginae

The results of the analyses for fluorine (Table 1) show quite clearly that very little organically-bound fluorine, as measured by the methods employed, was present in the fluoride-treated plants. Further, instead of synthesising organo-fluorine it is evident that, in those plants treated with fluoroacetate, this compound was largely degraded to an acid-labile form. Although the uptake of fluoride and

TABLE 1
FLUORINE IN EXPERIMENTAL *Acacia georginae* PLANTS

| | *Weight of fresh tissue (g)* | | *Weight of dry tissue (g)* | | *Fluorine (μg/g in air-dry tissue)* | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | *Leaf* | | | *Root* | | |
| | *Leaf* | *Root* | *Leaf* | *Root* | *Total* | *Acid-labile ('inganic')* | *'Organic F' in propan-1-ol* | *Total* | *Acid-labile ('inganic')* | *'Organic F' in propan-1-ol* |
| Ammonium fluoride in soil | | | | | | | | | | |
| S Fd 1 | 2·55 | 2·90 | 0·77 | 0·82 | 127 | 96 | 0 | 1077 | 1023 | 0 |
| S Fd 2 | 1·78 | 1·68 | 0·49 | 0·47 | 163 | 145 | nd | 1372 | 1315 | 0 |
| S Fd 3 | 3·99 | 4·40 | 1·48 | 1·39 | 69 | 68 | 3 | 1321 | 1300 | 4 |
| S Fd 4 | 2·08 | 1·86 | 0·63 | 0·53 | 116 | 114 | nd | 1290 | 1300 | nd |
| Mean | 2·60 | 2·71 | 0·84 | 0·80 | 119 | 106 | | 1265 | 1235 | |
| Ammonium fluoride in quartz | | | | | | | | | | |
| Q Fd 1 | 4·09 | 5·51 | 1·54 | 1·47 | 192 | 194 | 0 | 3376 | 3142 | 0·5 |
| Q Fd 2 | 3·67 | 4·19 | 1·77 | 1·40 | 265 | 264 | nd | 2784 | 2550 | nd |
| Q Fd 3 | 3·23 | 4·63 | 1·32 | 1·92 | 456 | 431 | 4 | 2700 | 2370 | 3 |
| Q Fd 4 | 5·28 | 6·93 | 1·89 | 2·22 | 172 | 172 | 3 | 3290 | 2980 | 1 |
| Mean | 4·07 | 5·32 | 1·63 | 1·75 | 271 | 265 | | 3038 | 2761 | |
| Sodium fluoroacetate in soil | | | | | | | | | | |
| S Fa 1 | 5·37 | 4·21 | 1·57 | 1·45 | 133 | 56 | 56 | 355 | 333 | nd |
| S Fa 2 | 4·15 | 4·91 | 1·34 | 1·48 | 159 | 76 | nd | 724 | 732 | nd |
| S Fa 3 | 8·64 | 6·28 | 2·64 | 2·13 | 95 | 43 | 44 | 431 | 378 | 5 |
| S Fa 4 | 7·21 | 5·25 | 2·27 | 1·71 | 160 | 31 | 104 | 640 | 605 | 32 |
| Mean | 6·34 | 5·16 | 1·95 | 1·69 | 137 | 52 | | 538 | 512 | |
| Sodium fluoroacetate in quartz | | | | | | | | | | |
| Q Fa 1 | 4·90 | 4·50 | 1·71 | 1·65 | 282 | 73 | 73 | 4855 | 4650 | 13 |
| Q Fa 2 | 4·89 | 7·07 | 1·62 | 2·13 | 127 | 127 | nd | 3324 | 2945 | nd |
| Q Fa 3 | 6·50 | 8·08 | 2·17 | 2·42 | 93 | 88 | 8 | 7450 | 5550 | 9 |
| Q Fa 4 | 4·23 | 3·45 | 1·40 | 1·19 | 160 | 30 | 144 | 6340 | 5450 | 33 |
| Mean | 5·13 | 5·78 | 1·70 | 1·85 | 166 | 80 | | 5492 | 4649 | |
| Pooled, untreated controls in quartz | | | | | 33 | 15 | 0 | 32 | 29 | 0 |

nd = not determined.

fluoroacetate by the plants was considerable in both growth media, much more acid-labile fluoride was found in the roots of the plants established in quartz than in those growing in soil.

The roots of the fluoroacetate-treated plants contained extraordinarily low levels of organic fluorine in relation to the total amount applied to the plants (approximately 300 mg of sodium fluoroacetate/plant).

The differences between the total and the acid-labile fluorine in the roots and leaves of the fluoride-treated plants grown in soil were relatively small but were greater in the roots of the plants grown in quartz. Much the same results were obtained with the plants treated with fluoroacetate when grown in soil, but the differences between the total and acid-labile fluorine in the roots of the fluoroacetate-treated plants grown in quartz were very much greater. It is difficult to interpret these results except to speculate that the differences between the total and

acid-labile ('inorganic') fluorine are more likely to be associated with the presence of silica affecting the diffusion of fluoride from the plant tissue than they are to be due to organic fluorine. The influence of silica in the determination of fluorine in plant tissue is well established (Remmert *et al.*, 1953). The much lower amounts of total and acid-labile fluorine found in the soil-grown plants are almost certainly due to adsorption of fluoride on to clay—a known property of clay soils (Bower & Hatcher, 1967).

*Fluorine in soils growing experimental* Acacia georginae

The total fluorine level of the control soil is typical of that normally found in a good arable loam and the increases in the treated soils are consistent with the amounts of fluorine applied (Table 2). The reversal of the amounts measured for total fluorine and acid-labile fluoride of the fluoride-treated soil may be due to sampling problems.

TABLE 2
FLUORINE IN SOILS GROWING EXPERIMENTAL *Acacia georginae*

| | *pH* | *Fluorine (μg/g air-dry soil)* | | |
|---|---|---|---|---|
| | | *Total* | *Acid-labile* | *Water-extractable** |
| Control soil | 5·9 | 257 | 170 | 2 |
| Control soil treated with $NH_4F$ (S Fd 1–4) | 6·3 | 1030 | 1040 | 142 |
| Control soil treated with fluoroacetate (S Fa 1–4) | 5·1 | 420 | 375 | 16 |

* The values are for total fluorine all of which was acid-labile.

The greatly increased acid-labile fluoride in the soil after treatment with fluoroacetate, combined with a significant increase in the water-soluble fluoride, almost certainly reflects metabolism of the organic compound either by microbial activity in the soil or by some function of the plant roots.

*Electrodialysis*

No organic fluorine was measured in the electrodialysate of the native soil used for cultivating the experimental *Acacia georginae*, nor was there any evidence of its formation after the application of ammonium fluoride (Table 3), but the dialysable inorganic fluoride, *i.e.* ionic fluoride, increased tenfold. The moderate amount of organic fluorine (3·5 μg/g) measured in the soil treated with sodium fluoroacetate was small compared with the total amount applied. No organically-bound fluorine was measured in the electrodialysate of a representative sample (15 g) of pooled dry whole plant tissue from four ammonium fluoride-treated specimens, although

TABLE 3
FLUORINE IN ELECTRODIALYSATES OF EXPERIMENTAL *Acacia georginae* PLANTS AND SOILS

| | *Fluorine (μg/g air-dry plant or soil)* | | |
|---|---|---|---|
| | *Total* | *Acid-labile ('inorganic')* | *Organic* |
| Control soil, no treatment | 3·2 | 3·2 | 0 |
| Soil treated with $NH_4F$ | 33·8 | 34·2 | 0 |
| Soil treated with fluoroacetate | 5·3 | 1·8 | 3·5 |
| *Acacia georginae* leaf, no treatment, grown in quartz | 2 | 1 | 1 |
| *Acacia georginae* (4 whole plants), treated with $NH_4F$, grown in quartz | 190 | 190 | 0 |

the dialysable fluoride was considerable. A very small amount of 'organic' fluorine was measured in the electrodialysate of the control plant tissue.

*Gas–liquid chromatography*

Monofluoroacetate was not detected in any of the electrodialysates of the control or fluoride-treated plants and soils.

*Infra-red spectroscopy*

There was no similarity between the stretching vibrations of the control and treated soils and those of sodium fluoroacetate or the tropical soils in which fluoroacetate had previously been established (Hall & Cain, 1972).

*The nature of the diffusible fluoride*

Notwithstanding that the extraction of the plants with alkaline propan-1-ol appeared to overcome, to a large extent, the difficulty of assessing the true organic fluorine, certain samples gave rise to puzzling results. The difference between the total fluorine and the acid-labile fluoride was very marked in the roots of those plants grown in quartz. In an attempt to explain these differences, direct reactions

TABLE 4
REACTIONS OF INORGANIC FLUORIDES AND PLANT EXTRACTS WITH LANTHANUM ALIZARIN COMPLEXONE

| *Sample* | *Fluoride measured (μg)* |
|---|---|
| Sodium monofluoroacetate (2·0 μg F) | 0 |
| Sodium fluoroborate $NaBF_4$ (2·08 μg F) | 0 |
| Sodium fluorophosphate $Na_2FPO_3$ (1·0 μg F) | 0·018 |
| 24 h reaction | 0·328 |
| 48 h reaction | 0·892 |
| Sodium fluorosilicate $Na_2SiF_6$ (1·14 μg F) | 1·082 |
| *Acacia georginae* (QFa 2 root; total F, 3324 μg/g) | 304 μg/g dry tissue |
| *Acacia georginae* (SFa 1 root; total F, 355 μg/g | 40 μg/g dry tissue |

using freshly prepared solutions of fluoroacetate, fluoroborate, fluorophosphate ($FPO_3$) and fluorosilicate with the lanthanum alizarin reagent (employed for the determination of inorganic fluoride) were measured, which revealed that of the four compounds only fluorosilicate reacted as fluoride. Potassium fluorophosphate was included as an example of a complex inorganic fluoride which is known to undergo slow hydrolysis in aqueous solution to ionic fluoride and reported to be formed in a biological system (Flavin *et al.*, 1957). Hot aqueous extracts were then prepared of the roots of two of the experimental fluoroacetate-treated plants (0·5 g in 10 ml) and aliquots not exceeding 0·4 ml (to avoid interference by organic acids) were reacted directly with lanthanum alizarin complexone. The reactions (Table 4) demonstrated that ionic fluoride was present, but it was a small percentage of the total acid-labile fluorine which must therefore have been present largely in an un-ionised form.

## DISCUSSION

No information has been published which indicates the mechanism involved in the synthesis of the carbon–fluorine compounds which are naturally present in some tropical plants. It is tacitly assumed that the fluorine is derived from the soil as an inorganic fluoride and that the enzymes of the plant are responsible for its conversion to fluoroacetate and higher carbon-numbered compounds. Mead & Segal (1972) have suggested a metabolic pathway via fluoro- ($C_3$-pyroxidal phosphate); their hypothesis is, however, without experimental evidence.

Several groups of workers have claimed synthesis of fluoroacetate in a variety of plants cultured experimentally, but in the work described here the application of ammonium fluoride to *Acacia georginae* did not induce the formation of any detectable fluoroacetate. On the contrary, it was found that the plant could metabolise this compound to an extraordinary extent, the fluoro-metabolite(s) being labile to perchloric acid under the conditions for the diffusion of hydrofluoric acid. Each of the plants grown in quartz was flushed with approximately 300 mg of fluorine as monofluoroacetate, yet relatively insignificant amounts of organic fluorine were measured in the roots. Since approximately 10% of the nutrient solution was retained in the quartz at each application, some 26 mg of organic F was potentially available to each plant. Of the total fluorine measured (22·62 mg) in the combined leaves and roots of the four plants, only 1·7% was organic whereas 84.5% was acid labile. The stems were not examined but, from previous analyses of native plants, it is probable that their total fluorine content was similar to that of the leaves (93–282 μg/g). Of all the organic fluorine estimated to be available to the four experimental plants in this group, nearly 22% was therefore absorbed, most of which was metabolised to another form and remained in the roots. Similar results were obtained for the plants grown in soil but, whereas the amounts of acid-labile

fluoride and 'organic' fluorine measured in the leaves of plants grown in both quartz and soil were of the same order, much less fluorine (total and acid-labile) was found in the roots of those grown in soil. These differences are probably due to adsorption of fluoride and fluoroacetate on to the clay fraction of the soil, making both compounds less readily available to the plant. Since neither the soil nor the nutrient solutions were sterilised in these experiments, some of the fluoroacetate may have been degraded to inorganic fluoride by microbial activity—micro-organisms capable of cleaving the C–F bond are now known to exist in soil (Kelly, 1965; Goldman, 1965; Cain *et al.*, 1968).

The age of the plants may be a factor in the bio-synthesis of carbon–fluorine compounds. Peters *et al.* (1965) have commented on the inability of old plants of *Acacia georginae* to synthesise organic fluorine but reported that fluoroacetate was formed in a homogenate of *Acacia georginae* seedlings after the addition of fluoride, pyruvate, adenosine triphosphate and manganese ions. They have reported similar observations in respect of single cell cultures of soya bean (*Glycine max*) grown on agar containing sodium fluoride and, further, that fluorocitrate may be formed in single cell cultures of *Thea sinensis* and in preparations of oatmeal (Peters & Shorthouse, 1972*a*, *b*).

The analyses reported here (Table 1) of the *Acacia georginae* plants treated with ammonium fluoride are not convincing evidence for the synthesis of organic fluorine, and gas–liquid chromatography failed to detect any fluoroacetate in the electrodialysates or propanolic extracts of the plant tissues even though quite large weights of sample (15 g) were examined for organic fluorine. Even the small amounts of organic fluorine which were measured must be regarded with caution since analytically the fluorine determinations were near the lower limit of the estimation (0·05 $\mu$g $F^-$).

One of the most difficult features of this work is the differentiation of true organic fluorine from inorganic fluoride. Various workers have estimated 'organic' fluorine in the plants they have studied as being the difference in the measurement of total fluorine after ashing, and acid-labile fluoride (*i.e.* diffusible, inorganic fluoride). But in fact, during this present investigation, the analyses of inorganic minerals and soils revealed that, after 10 days of diffusion, perchloric acid may still not release all the fluorine present (Hall & Cain, 1972) although virtually quantitative recovery of fluorine can be obtained from pure compounds of fluoroborate, fluorophosphate and fluorosilicate.

In the work described here emphasis was placed on the extraction into alkaline propan-1-ol for the determination of true organic fluorine. The procedure was devised to avoid interference from inorganic fluorides on the assumption that these would not be extracted. From previous studies (Hall, 1972) it is evident that 90% v/v propan-1-ol containing ammonium or lithium hydroxide quantitatively extracts the organic fluorinated compounds up to $C_{18}$ which are present in the tissues of certain tropical plants and in general does not extract the acid-labile fluorinated

compounds (including inorganic fluorides) of these same species or of experimental *Acacia georginae* leaves and roots. When it is considered that the amounts of fluorine measured in the roots of the experimental *Acacia georginae* plants were exceptionally high, the correlation of acid-labile fluorine with total fluorine is reasonably close—particularly in the tissues of the fluorinated plants grown in soil (Table 1). The results of these analyses, taken with those of the electrodialysate of the fluoridated *Acacia*, suggest that the acid-labile fluorine in these tissues was either inorganic fluoride, or fluoride loosely conjugated to an organic moiety, *e.g.*, protein or carbohydrate. The larger differences between the total and diffusible fluorine in the roots, and in a few examples in the leaves, are due, it is thought, to the effect of silica in forming inorganic fluorocompounds which are relatively resistant to degradation by 35% w/w perchloric acid under the conditions used for the diffusion of HF, and not to the presence of fluoroacetate and related compounds which are extremely stable to concentrated perchloric acid at 60°C but which would be extracted into the alkaline propan-1-ol. Similar observations have been made with tissues of toxic tropical species especially of *Dichapetalum stuhlmannii* (Hall, 1972). The total fluorine in a specimen of the dry root tissue of this plant was 1916 μg/g, of which only 18 μg/g was organic, yet 476 μg/g remained undiffused after 5 days with 35% w/w perchloric acid and 216 μg/g remained even after 10 days. It is felt, therefore, that the difference between the total fluorine and the diffused (acid-labile) fluoride cannot be regarded as a measure of the true organically-bound fluorine.

The finding that a simple hot water extract of the roots from the fluoroacetate-treated plants gave a positive reaction with alizarin complexone (alizarine fluorine blue) is convincing evidence of degradation to inorganic fluoride but represented only 10% of the total acid-labile fluoride. What then was the remaining acid-labile fluorine? If it was the common fluorosilicate $SiF_6$ it would have reacted with the complexone. It is possible that a more stable fluorosilicate had been formed or a fluorophosphate, or an acid-labile fluorocarbohydrate of the kind described by Barnett *et al.* (1966) and by Kent (1969).

It seems that more knowledge is required of the basic physiology associated with fluoride metabolism in plants. Attempts to elucidate the character of inorganic, as well as organic, entities of fluorine in plant tissues which have accumulated the element might help to explain the synthesis of naturally-occurring carbon–fluorine compounds. Whilst it is possible that small amounts of organically-bound fluorine may be formed in common plants when growth is subjected to unusual ecological conditions, the experiments described here indicate that such formation is not easy to induce artificially. Pollution of the environment and its effects must be under constant surveillance but it seems unlikely that the levels of toxic C–F compounds in pastures, arising from the sources of industrial contamination mentioned earlier, if they are formed at all, constitute a serious hazard. It is probable that many environmental factors are involved in the synthesis of carbon–fluorine compounds in plants

and a thorough investigation of the plant/soil relationship, with special emphasis on the root and soil microbial systems, could well prove rewarding.

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