SPECTROCHEMICAL STUDIES ON THE UPTAKE OF IONS BY PLANTS

I. The Lundegårdh Flame Technique of Ash Analysis of Toxin/Antibiotic Invaded Cotton Plants

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Introduction

The Lundegårdh flame technique has been widely used for many investigations, as a flame emission spectrochemical tool for studying the distribution of many metallic ions in biological materials (Hultgren, 1932; Mitchell, 1948 and Lundegårdh, 1951). In this laboratory this technique has been standardised for the air-acetylene flame and used as a routine method in the investigation of ashes of both healthy and fungal root diseased plants, as also fungal ashes grown *in vitro* in synthetic nutrient substrate.

Using cut shoots of plants, for studies on toxin uptake and the consequent chelation with metallic ions, has been a standard method in most laboratories (Waggoner and Dimond, 1953; Gäumann and Naef-Roth, 1954, 1955 and 1956 and Gäumann, Naef-Roth and Kern, 1955). But in the results presented here, entire cotton plants grown in natural soils and soils infected with *Fusarium vasinfectum* Atk., have been studied instead of excised shoots. The importance of this investigation, hardly needs any favourable endorsement, as the mechanism of wilt produced by *vivotoxins* has remained obscure, despite much work on enzyme systems of the pathogens and also work on host physiology, especially on transpiration.

It was earlier indicated by one of us (Sadasivan, 1955), that toxins/antibiotics seem to interfere with enzyme systems and produced derangement of key metabolite(s). The present study of alkali and alkaline earth metals and iron and manganese and their distribution in healthy and diseased cotton plants was taken up to elucidate this postulate further.

Materials and Methods

Soil, pathogen and host.—Culture of *Fusarium vasinfectum* Atk., supplied by Centraalbureau voor Schimmelcultures (Baarn), was used to infect cotton plants, commonly called Karunganni 2 (*Gossypium arboreum*) grown in sterilised garden soil. Sterilisation of the soil and methods of artificial inoculation were as detailed previously (Sarojini, 1951).

Preparation of ash samples.—1 g. of oven-dry, finely ground plant samples were ashed for 8 to 10 hours at about 450° C., then taken to dryness twice with concentrated HCl on a steam-bath. This was then taken up in 5 to 10 ml. of 2 N HCl, filtered and made up to 50 ml. This formed the basic concentration for all analyses.

Standard solutions.—As is well known the Lundegårdh flame technique has the advantage, in that it carries duplicate spectrograms of the standard solution (Ca M/4,000; Sr M/10,000; Mg M/200; Na M/100; K M/200; Fe M/200; Mn M/4,000; and Li M/4,000) as well as its five dilutions, in N/20 HCl (Analytical reagent) on the same plate as neat, one-half, quarter, three-twentieths, one-tenth and one-twentieth and also spectrograms of unknown sample solutions.

The equipment.—A Hilger medium quartz spectrograph with Judd Lewis comparator and a non-recording microphotometer (mains operated and with a nickel/cadmium NIFE battery on floating circuit) formed the main equipment. The Lundegårdh flame was fed with acetylene at 20 ml. water manometer pressure and compressed air from an air compressor at 25 lb. per sq. in. A vent-axia fan at the end of a hood kept the flame steady (Mitchell, 1948). An A.C. spark between copper electrodes at 15,000 volts using $\frac{1}{4}$ K.W. transformer (F.S. 201 Hilger), $\frac{1}{4}$ K.W. condenser (F.S. 202 Hilger) and self-induction coil (F.S. 203 Hilger) and 40 ohms resistance was passed through the flame just above the blue cone (Hultgren, 1932). The self-inductance tapping was at 0·5 millihenries. This spark-in-flame increased the sensitivity of Mg, Fe and other alkali metals. Slit-width of 0·04 mm. was used throughout and the wedge adjusted to 1·5 mm. Both Ilford Zenith supersensitive and Ilford Q2 plates (10″×4″) were used for the photographs. The standard and the test solutions were sprayed from the Lundegårdh glass sprayer vessel as usual. Exposures were at 60 seconds throughout and the plates were developed at 20° C. for the recommended times in the proper developers. Photometry was done after spotting the lines on the comparator (Mitchell, 1948). The lines chosen for the elements under study were as follows:—K—4044/47 Å (as a doublet); Na—3302 Å; Mg—2852 Å; Ca—4226 Å; Mn—4030/33 Å (as a doublet) and Fe—2719 Å, (M.I.T. Tables). Although the most sensitive lines of K are the 7664/98 Å, the 4044/47 Å lines in the UV region were used as they were found to be quite satisfactory and obviated the use of Ilford Long Range spectrum plates for this element alone.

EXPERIMENTAL

Solutions of ash samples of 15-day old healthy and *F. vasinfectum* infected cotton plant shoots were carefully prepared as already detailed and

aliquots of these were sprayed into the flame (spark-in-flame). The spectra of the standard solution and its five dilutions preceded the spectra of the unknown samples. The solutions of the plant samples were further diluted for the determination of Ca, as the quanta of Ca present in the neat standard solution was much less than that occurring in 15-day old plant ash solutions of the basic concentration (*vide* Materials and Methods). The spectra were spotted for the lines of interest and read in the microphotometer. The transmissions of the lines in the spectra of the standard solutions, as seen by the galvanometric deflections was recorded and plotted to give the typical Lundegårdh working curve of the ratio of the galvanometer deflections of the line *plus* background, to background, plotted against concentrations. Similarly, the values of the lines of the unknown solutions were taken and juxtaposed on the standard curves and the quantitative estimations of the unknown samples arrived at. Table I summarises the results and gives the values for each element as p.p.m. or mg./g. dry weight.

TABLE I

The amount of K, Na, Mg, Ca, Mn and Fe in ash samples of 15*-day old cotton plants infected by* Fusarium vasinfectum

Results expressed as p.p.m. and mg./g. dry weight of plant tissue

	Dry weight in g.	Ash weight in mg.	K		Na		Mg		Ca		Mn		Fe	
			p.p.m.	mg./g.	p.p.m.	mg./g.	p.p.m.	mg./g.	p.p.m.	mg./g.	p.p.m.	mg./g.	p.p.m.	mg./g.
A ..	1·0	120·0	173·9	10·4	11·5	0·7	69·3	4·2	250·3	15·0	3·0	0·2	4·2	0·3
B ..	1·0	82·0	7·7	0·3	10·3	0·4	167·2	6·9	451·0	18·6	13·3	0·5	11·2	0·5

A—Healthy. B—Infected.

DISCUSSION

The critical period for the appearance of "vein clearing" symptoms in cotton seedlings from the time of germination, grown in *F. vasinfectum* infected soils has been variously stated as 10–15 days (Kalyanasundaram, 1954). The present set of infected and healthy plants were accordingly collected at the end of 15 days for ash analysis. It is obvious from Table I that K content in the infected plants goes down very considerably and, indeed, is most conspicuous possibly by virtue of its extreme mobility in the healthy host, where rapidity of entry is unhampered, whereas in the infected condition there obviously exists an osmotic barrier resulting in loss in permeability to a large extent to this particular element. The other alkali

metal Na shows very slight decreased accumulation in the infected over the control. On the other hand, the alkaline earth metals Mg and Ca behave dissimilar to the alkali metals inasmuch as they show increased quanta in the infected host tissues. The heavy metal Mn shows a very remarkable rise in quantity in the infected host tissue, a fact which is of considerable significance as it is known to chelate very readily with fusaric acid and might have been translocated as a chelate complex. Fe also registers an increase in the infected plant tissue, though not as spectacular as Mn. It is not clear, as to what significance is to be attached to the higher uptake of Fe and Mn by the infected plant, although it has to be stated that the Fe/Mn ratio of 4·2: 3·0 p.p.m. in the healthy is in the order of 11·2: 13·3 p.p.m. in the infected plant tissues. It may be that the uptake of essential K as also Mg and Ca have a bearing on the ratio of Fe/Mn. Earlier work in this laboratory (Varadarajan, 1953) had indicated that addition of Fe/Mn to *F. vasinfectum* infested soils in the ratio of 1 : 2 (40 p.p.m. Fe/80 p.p.m. Mn) did bring about a recovery on the 20th day from the wilt symptoms which were very prominent during the 13–14-day periods, after germination of the cotton plants.

It is not our intention at this stage to enter into any elaborate analysis of the causes of the wilt, but it may be pertinent to state that the present data clearly indicate a primary loss in permeability of the tissues with the onset of toxin invasion resulting in very considerable derangement (loss) of the entry of the key metabolite K and an accumulation of varying quantities of Mg, Ca, Fe and Mn. This deranged selective absorption or imbalance in ionic uptake as seen here, appears to lend less and less support to the vessel plugging theory (Scheffer and Walker, 1953) of Fusarial wilts, which may have to be discarded in the light of loss in cell functions in the root region and consequent deranged osmosis, now implied by this investigation.

SUMMARY

Spectrochemical analysis, using the standard Lundegårdh flame emission (spark-in-flame) method, of cotton plants infected by *Fusarium vasinfectum* Atk., showed an increased uptake of Mg, Ca, Fe and Mn with decreased accumulation of K over the healthy plants. It is suggested that the derangement in the selective absorption of ions, seen in the infected plants, is more likely to have been caused by chemical agents (toxins) than physical causes such as plugging of vessels.

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