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▶▶▶ February 2015 ◀◀◀

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Investigations relating to the persistency of herbicides

Experimental procedures for sampling and  
determination of residues

John Fryer and I have been discussing with Hance and other staff at the Weed Research Organization what steps in procedures and techniques can be taken to limit the number of soil and leaf litter samples which can be analysed for herbicide residues at I.R.R.I. and yet at the same time allow a reasonable programme of field work to be undertaken.

At the outset it must be re-emphasized this evaluation must have a high degree of uncertainty since prior knowledge of the problems involved in the tropics is restricted and experience for temperate conditions either makes prediction uncertain or not possible because of totally different conditions e.g. mangrove swamps.

On the basis of experiments in England, statistical evidence indicates that for the size of plot envisaged in the investigations there should be at least 20 cores or samples per plot. If a similar sampling rate was adopted for the programme outlined in 'Preliminary Proposals for Studies on the Persistence of Herbicides in Forest and Mangrove Soil' than, allowing for subdivision of the cores, the number of analyses required without bulking would be in excess of the number which I.R.R.I. could be reasonably asked to undertake.

The proposals for the reduction in the number of analyses are as follows:

I(a) Sampling. For the initial sample to determine the rate of deposition of the spray, 20 Whatman chromatographic paper strips (45.7 x 7.6 cm) should be placed in their aluminium holders at random on the plot. Immediately after spraying each strip should be folded and rolled with the receiving surface on the inside and secured with a rubber band. The 20 papers should then be placed in a double plastic bag and transferred as soon as possible to a deep freeze. Also prior to spraying, surface soil samples - say five per plot - should be taken by means of cylindrical tins, of known diameter and depth, and sealed and transferred to a cold store. These tins would not be chemically analysed but

used to determine the total weights and the water content of the soil so that the herbicide reaching the soil surface can be expressed as p.p.m. on a dry weight basis.

Since with the orange agent it is possible to incorporate an oil soluble dye, the analysis of the amount of deposit per  $\text{cm}^2$  could be obtained by elution with an appropriate organic solvent and the amount in solution determined with a colorimeter.

For agent white with its water soluble components, the papers should be extracted and analyzed by G.L.C. procedures for 2,4-D or picloram. The alternative of using the dye tartrazine which is water soluble is being examined.

In the mangrove experiments, since the soil surface will be wet, a narrow strip of wood should be placed between the aluminium holder and the soil surface.

I(b) Bulking. As the aim is to measure the mean initial rate of deposition, it seems pointless to analyse each of the paper strips. Bulking into two lots of 10 would probably suffice and bulking into four lots of five ample.

II(a) For all samples subsequent to establishing the initial level of deposition, the procedures will be dependent on the type of experiment and the type of agent. In the mangrove area on each occasion there will be 20 surface samples by tin and 20 soil cores to a depth of 75 cm. With agent orange there will be no division of the core but with agent white, with its greater mobility in the soil, it is proposed that each 75 cm core will be divided into two.

For the surface sampling using an open ended tin, it will be necessary to punch a very small hole in the bottom of the tin to allow the air to be expelled when the tin is pushed into the soil and to stop the moment any water or mud exudes through the hole. The bottom of the tin should then be wiped dry in situ and the hole covered with sealing tape, of which there is a large amount in the accessories brought to Saigon with the first soil sampler. To withdraw the tin without loss of contents, the simplest procedures would appear to be to push in a narrow spade adjacent to the tin and (i) put a hand over the bottom of the tin

before pushing it out and cleaning off the excess mud or (ii) to insert a thin piece of metal parallel to the bottom edge of the tin and then prise the tin out and clean up. At the mangrove sites a bucket for water and a supply of rags or paper towels will be needed.

If it is feasible to undertake a further experiment on a 'forest soil' then sampling should be done by taking soil cores and dividing each core into three if conditions allow sampling to 75 cm.

**Bulking.** It is again proposed that each of the surface samples, the intact cores or other segments of cores, should be bulked in twos. Much discussion has taken place about the methods of bulking, particularly for the mangrove soils. With the original soil samples from Vietnam collected in October, work at WRO has shown that hydrolysis of the ester is complete within 14 days at the most. Since we are now proposing to estimate the initial deposit in the soil by use of filter papers, and all subsequent sampling will occur after the herbicide has been in the soil for at least three weeks, no case can be made out for taking precautions to prevent loss of 2,4,5-T vapour as the ester. The acid is not appreciably volatile.

It was also found that the efficacy of the extraction of 2,4,5-T was much higher under acidic as against alkaline conditions, while for the extraction both 2,4,5-T and of picloram the amount of alkali was critical if interfering peaks were to be avoided. It therefore seems clear that the use of Na OH pellets involves too many uncertainties and that alternative means should be considered.

John Fryer's letter of 10th February indicated that McKone, the analyst at the WRO, had put forward that by the use of glacial acetic acid in the preparation of the slurries, the pH could be reduced to a level inhibitory to most organisms in the soil. Further thought raised objections. Acidification could only apply to the orange samples and not to the samples containing residues of picloram. There would be problems of storage of acidic slurries. Mangrove soils will be highly buffered, so excessive amounts of acetic acid would have

to be added to maintain a pH low enough to be inhibitory. It seemed therefore that a much more inhibitory agent was required whose addition is unlikely to interfere with the analytical procedures and that 40% formalin met these requirements. Subsequent reference to the literature suggested that to each 250 ml of slurry 1 ml of 40% formalin should be added. There was the further advantage that the formalin treatment could apply to soils receiving both orange and white agents.

Against this background the following procedures are now advanced for bulking. Because of the high clay content of mangrove soils efficient mixing of samples will only be achieved by making a slurry using plastic buckets or large 1000 ml beakers and a paint stirrer attachment to an electric hand drill. Tests on a heavy clay soil suggest that an amount of water equivalent to half the weight of soil will be required for successful sub-sampling. The sub-sample of slurry which will allow adequate checking of extraction and analytical procedures, that is containing at least 150 g of dry soil, will be of the order of 250 ml to which should be added about 1 ml of 40% formalin to inhibit attack by micro-organisms.

The results of the analyses may be expressed conveniently in terms of ppm dry weight of soil. At the time of analysis a sub-sample of the soil slurry should be analysed for moisture content. This avoids any recording of weight of samples or volumes of water added during the bulking of the samples.

The preparation of the slurries will require simple laboratory facilities and equipment which apart from the stirring device already mentioned should include large beakers, measuring cylinders, (1000 ml, 250 ml and 5 ml), calibrated pipettes with a safety bulb filler and rough balances covering a range of a kg downwards.

Between the time of sampling in the field and bulking the tins from the surface samples should be stored temporarily in a deep freeze. The intact or sub-divided cores could be collected in double plastic bags or in the plastic

containers with screw tops which are 'over' from the original purchase made in Saigon. The plastic containers should be large enough to allow at least pairs of segments if not intact cores to be put into a single container during sampling in the field before storage in a deep freeze. *W. V. ...*

In order to cut down the requirements for cold storage bulking should take place as soon as is feasible after sampling in the field. This is particularly desirable and storage of the slurry should not be in tins unless the slurry can first be placed in a sealed plastic bag. Since the volume will be 250 ml or more the stock of paint tins, unless they are of a half pint size, will not be big enough. It would be far better to store the slurry samples in plastic bottles with water tight screw tops and a minimum capacity of 250 ml. X

III. Collection and analysis of leaves. The plans for applying both orange and white agents by helicopter to stands of mangrove include the collection of falling leaves 1, 2, 4, 8 and possibly 16 weeks after spraying to determine (a) the amount and rate of litter fall and (b) the amount of phytotoxic compounds which are returned to the soil in the fallen leaves.

As with determinations of the measurement of deposition on the soil using paper strips, the essential information is to determine at each sampling date the mean amount of leaves deposited per unit surface of soil and the average amount of residues contained in the leaf litter. Thus the mean values for residues can be achieved by sub-sampling the litter trays and bulking for a final sample for measuring water content, storage and analysis. At the same time some untreated leaves should be plucked and collected and dried to serve for 'blank' determinations after drying.

The simplest procedure would be that for each compound and for each sampling date, known weights would be transferred to double plastic bags and immediately placed in a deep freeze for subsequent analysis. The leaf sample and analysis for each combination of agent and date should be duplicated but for all unsprayed leaves only a single sample is required.

IV. Maximal requirements for analysis. On the basis of the foregoing sections, an estimate of the total analytical requirements can be made. The data on persistency in the soil will include analyses of paper strips or soil samples and the characteristics of the soil samples for weight, water content and soil volume, or controls to check analytical procedures.

It has been proposed that (i) there should be 20 soil or paper strip samples per plot per date, (ii) that surface samples (tins) or deeper samples (cores) should be bulked in pairs making groups of 10 samples per plot, (iii) analyses of paper strips should be in duplicate or possibly quadruplicate, (iv) the control samples will be of two kinds. In one where the estimates are physical and the samples only require being kept in cold storage and in the other where to ensure comparability in the analyses of the herbicide components, slurries of the controls with the addition of formalin, will be required. (See Table 1)

Estimates of the total number of samples are set out in Table 1. To reiterate some of the criteria it has been advanced that in the mangrove experiments sampling for orange in the field will consist of one tin and a core to 75 cm, while for picloram besides one tin the core should be divided into two (or three if possible). In the suggested forest soil experiment in Vietnam it is presumed that it follows the procedure at I.R.R.I., namely sampling will be by cores and each core is divided into three.

In estimating the number of samples at I.R.R.I. if the existing proposals are adopted, there will be no need to analyse the initial shallow cores to 25 cm since the initial dose can be estimated from the paper strips so the 22 samples can be deducted but the control cores will be needed to estimate the weight and water content and whether there is interference in the analyses. On the basis of the original plan see preliminary record 'Persistence of Herbicides 'Orange' and 'White' in a Forest Soil' no blanks have been included in the subsequent samples and presumably Newton in the second sampling did not do so. If, however, bulking and the addition of formalin is accepted, then blanks for control slurries should be taken in the subsequent sampling decisions.

Table 1

Estimates of bulked samples for analysis for both agents in the individual experiments

Sampling occasion (weeks)	Surface (tins)	Soil Samples			Paper strips	Leaves
		Control	Cores	Control		
<u>Mangrove - helicopter</u>						
0	0	0	0	0	8	0
1	20	4	30	6	0	6
2	20	4	30	6	0	6
4	20	4	30	6	0	6
8	20	4	30	6	0	6
16	20	4	30	6	0	6
					Total	338
<u>cleared Mangrove -/ground spraying</u>						
0	0	0	0	0	8	0
3	20	4	30	6	0	0
9	20	4	30	6	0	0
27	20	4	30	6	0	0
					Total	188
<u>Forest - soil</u>						
0	0	0	0	0	8	0
3	0	0	60	8	0	0
9	0	0	60	8	0	0
27	0	0	60	8	0	0
					Total	212
<u>Forest - soil I.R.R.I.</u>						
0	0	0	22	4	8	4
3	0	0	60	0	0	0
9	0	0	60	0	0	0
27	0	0	60	0	0	0
					Total	218



It might be advanced that the provision of the blank samples is over generous. If the earliest samples show no interference in the analyses the later ones could be discarded in the laboratory.

Lastly it is to be noted that the total number of residue analyses for all the experiments set out in Table † does not exceed a 1000.

From the estimates it is also possible to calculate the total requirements for tins, plastic bags, screw top containers and plastic bottles for the final samples of slurry.

In Vietnam the requirement for tins for surface samples is just under 400 which is, I believe, the original number sent jointly to Vietnam and the Philippines; the number will require checking and the tins collecting together in Vietnam. If only plastic bags are used for the collection of individual samples prior to bulking then the requirement is about 2000. If direct bulking into the stock of plastic containers with screw tops is used for the larger samples (i.e. intact cores with a requirement of c. 100 containers) there will be a small reduction in the number of bags.

For the final storage of slurries containing 4% formaldehyde the total number of wide-mouthed plastic bottles with screw top containers and a capacity of 250-300 ml will be about 750 and for the Philippines about 200.

V. Application by Helicopter. In planning the helicopter spraying additional information is required. In the first place are helicopters already fitted with spraying equipment immediately available? There is a need to check their performance and rate of output from the boom both by direct measurement on the ground and from the air. Measurement in flight can be done by adding dye to agent 'Orange', flying the helicopter (at the height likely to be used in the mangrove area) over an aerodrome where Whatman paper strips are set out at right angles to the flight path. How far away will be the aerodrome where the herbicides are taken on board and what would be the estimated time to start and complete the mission? Again how long is required to empty the spraying

gear of one compound and flush out the system with a cleansing fluid before filling with the second? Since the best conditions for flying are in the early morning it may be that only one compound can be sprayed and sampled in a day. This in turn raises queries of curfew regulations on travel since it may be necessary to put down the paper strips at dawn. Lastly the tidal position requires very careful consideration to ensure that spraying can be done after the tide has receded from the surface and enough time is left for sampling. Fortunately on the basis of the new plan there will be no requirement for initial soil samples. But putting down and picking up the paper strips in a mangrove 'thicket' will be time consuming. Phil Ross has already been written to about the need for a narrow path down the middle of each strip.

From many aspects there is everything to be said for not attempting to apply both herbicides on one day.

VI. Tailpiece. According to the provisional programme for a return visit of members of the Committee in March, the mangrove experiments are proposed for the first week of March after Newton has visited the Philippines. In view of all the queries raised in this working plan about facilities and arrangements, it does not seem logistically probable that all the requirements can be met so early in the month. Since the time of low tide is a vital factor postponement towards the end of the month may be essential.

G.A. Blackman

February 1972