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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' then, 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 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 tins 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 of both 2,4,5-T and 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.

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.

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 Control	Cores	Control	Paper strips	Leaves
Mangrove - helicopter						
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	
Mangrove - cleared/ground spraying						
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	
Forest - soil						
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	
Forest - soil I.R.R.I.						
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 1 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 their 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 reised 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

NAS Committee on the effect of Herbicides in Vietnam

PRELIMINARY PROPOSALS FOR STUDIES ON THE PERSISTENCE OF HERBICIDES IN FOREST AND MANGROVE SOIL

J. D. Fryer

G. E. Blackman

INTRODUCTION

When herbicides such as 2,4,5-T and picloram are sprayed from an aircraft on to forest only a small proportion will reach the soil direct. After emission from the spray nozzle some of the herbicide will be lost before reaching the canopy as a result of drift of small droplets out of the target area or volatilisation. The majority will be retained on the foliage of the forest plants and then either absorbed or left on the leaf surface. Of the fraction entering the leaf, some will be metabolised within the plant and some reach the soil when dead leaves, twigs or branches fall from the tree.

It may be expected, therefore, that considerably less than the nominal 3 gal/ac of the defoliant agents used in Vietnam will have reached the soil either directly or indirectly. The maximum levels will have been reached in areas where previous sprayings, forest clearing or agriculture, had greatly reduced the amount of vegetation cover. These levels may have approached the nominal dose in open places and occasionally exceeded it when overlap of spray swathes occurred.

The proposed main experiments are designed to investigate the rate of disappearance of 2,4,5-T, picloram and 2,4-D - in order of priority - when a dose of 3 gal agent/ac is applied direct to the soil. Under these conditions residues in the soil are likely to be greater than those generally resulting from the defoliation programme due to the elimination of some of the routes of loss already described. However, it should be borne in mind that where it is necessary in the case of upland forest or mangrove experiments to remove undergrowth from or clear-fell a site prior to spraying to allow reasonably uniform application of the herbicides there will be difficulties in the initial dose and the rate of disappearance of residues compared with the treatment of a relatively undisturbed forest. For this reason it is hoped that in Vietnam it will prove possible to arrange, in addition, aircraft applications of 'orange' and 'white' to allow assessment of the persistence of herbicide residues following an initial treatment of at least an undisturbed mangrove area and if possible a forest area also. The latter may, however, well prove impossible.

Experiments are also proposed to examine the effect of 4 doses of the agents on agricultural soil on establishment and subsequent early growth of a range of crops.

PROPOSED INVESTIGATIONS

The persistence of agents 'orange' and 'white' in mangrove and forest soils in the Phillipines and Vietnam.

I MANGROVES

It is hoped that the following experiment will be carried out in the Phillipines and, as far as possible, duplicated in Vietnam.

Object: To determine the rate of disappearance from soil of 2,4,5-T, picloram and 2,4-D applied shortly after clear-felling an area of mangroves.

Site: This should be at the higher end of the tidal range to allow adequate time for spraying and soil sampling when the mud is not covered by water.

Treatments: Orange

C

3 gal (US)/ac
= approx. 12.9 lb 2,4-D a.e./ac + 13.2 lb 2,4,5-T a.e./ac

White

C

3 gal (US)/ac
= approx. 1.6 lb picloram a.e./ac + 6.0 lb 2,4-D a.e./ac

Spray volume: Approximately 30 gal/ac according to equipment available and results of test spraying.

Formulations: 'Orange' is a rather viscous oily fluid which must either be diluted with oil or emulsified with water after the addition of a suitable emulsifier, before it can be applied at the desired rates by conventional plot spraying equipment. Diesel or kerosene are suitable diluents but might be objected to on the grounds that at high rates they might conceivably alter the rate of disappearance of the herbicides. If an oil/water emulsion is used, the possibility of the emulsifier having an effect on dissipation must also not be overlooked, e.g. by allowing greater mobility of the herbicide in tidal waters - a problem that could be overcome by having a quick-breaking emulsion. The advice of Dr. C. Minarick is being sought.

Plot size and layout: To be determined on site but for the basis of planning it is suggested that an area not less than 90 x 30 yd be cleared to allow 3 x 30 x 30 yd plots, two to be treated with i) orange ii) white; the third as control. Stumps should be cut as near ground level as possible to facilitate spraying. Trash should be removed from area and could be stacked around perimeter. It should not be burned near the site as charcoal could confuse the results. To minimise the risk of cross contamination the cleared area should be orientated so that the main flow of tide is parallel to its shorter sides. If due to local topography it is not possible to have the 3 plots all in one block, each can be located in a separate clearing, in which case the sites should be as close together as is feasible.

Plot marking: Some way will have to be found to locate accurately each plot for the duration of the experiment. Permanent markers for each plot corner will be needed. Unless the area is securely guarded at all times, the removal of markers by local people must be anticipated. If the plots are located by triangulation to adjacent uncut, marked mangrove trees, care will have to be taken that these will still be identifiable in the event of their being cut down by local people.

Tide at time of spraying: Spraying should take place on the ebb tide after the water has drained from the plots. Provided the chemicals have been prepared and measured beforehand and the spraying equipment is in good order and has been calibrated, it should be possible to spray both treatments within 2 hours. Dr. Chandler and Professor Ho are requested to advise urgently on days in January when low tide occurs around noon in the selected areas in the Phillipines and the Rung Sat, Vietnam, respectively.

Precautions for spraying: It is suggested that the spray liquids are strained through a fine mesh filter whilst being poured into the sprayer. They should be made up into transparent containers to allow any crystallisation of 2,4,5-T - ester or emulsion - breaking to be detected. Unless previous tests confirm that adequate stability of an oil/water emulsion of 'orange' can be assured for the required time to allow detection of concentrate in the laboratory, mixing up must be done on site and jerry cans of clean water will be required (total 20 gal?). To prevent drift of spray on to adjacent plots (this is particularly important in view of the high doses used), two windshields may be required - one up-wind, one down-wind. Hessian stretched over a light wooden frame (about 2.5 x 5 ft) is much better than plastic film for this purpose. To assist accurate spraying, a stop watch should be available.

Spraying equipment: Note: The spray bar (with pressure guage) and nozzle set-up should have been previously calibrated with the spray liquids to be used to provide reasonably uniform deposit over the spray swathe. The viscocity, throughout and spray fan angle may be appreciably different for whatever formulation of 'orange' is used and for 'white' - and even for the different concentrations. It is essential that a full calibration programme is completed before reaching the site and if necessary different spray bar and nozzle configurations provided.

Tests at WRO point to use of a Polyclair Knapsack sprayer fitted with a special lance and sprayboom. Promising results have been obtained with Allman fan jets operating at 10 p.s.i applying 30 gal/ac at 2 m.p.h. to a 2 yd swathe. The present plan is to take out this equipment to the Phillipines, use it for the mangrove and forest sites selected by IRRI and then take it to Saigon. If IRRI does not have suitable equipment, WRO can make available two sets - one for Vietnam, one for Phillipines.

Sampling for residues: Schedule. Suggested times of soil-sampling are: 0, 3, 9, and 27 weeks after treatment. It is necessary to sample immediately after spraying (shallow cores only - say 2 in.

deep) and before the plots become flooded by the tide to estimate the percentage recovery of the applied herbicide. This may vary from around 60 to 100% + according to previous experience in agricultural soils. If possible not less than 20 cores from each sprayed area should be taken at random from each treated area at each sampling date. Without preliminary work to measure variability of residues, there can be no statistical basis for choice of number of cores per plot and logistics are likely to prove the deciding factor. At the 2nd, 3rd and 4th sampling dates, cores should be taken to the full depth of the corer (c. 75 cm), each core being divided into the top 5 in. (12.5 cm) and two 26 cm sections. On each sampling occasion, 5 comparable random cores should be taken from the untreated area. The core sections should be placed in separate polyethylene bags, labelled and stored in a 'deep freeze' within a few hours of sampling until required for analysis. If 20 samples are taken from each treated area and 5 from the control, the number of samples for analysis will be 20 x 1 x 2 + 5 = 45 from the first sampling date and 20 x 3 x 2 + 5 x 3 = 135 for each of the three remaining dates, i.e. a total of 450 samples. WRO experience suggests a maximum of 16 determinations of 2,4,5-T a day or 8 - 10 picloram per GLC unit. If it is necessary to reduce the number of samples by bulking and sub-sampling, it must be realised that it is unlikely to be easy with this type of soil and that the mixing process will result in aeration of the samples and possibly a rapid change in pH and the microbiological status of the soil, which might affect the persistence of the herbicides. *

Equipment: The WRO dual purpose 2 x 36 in. soil corer is already available in Saigon suitable for both forest and mangrove soils. A new light-weight corer is currently being constructed at WRO which should be suitable for the mangrove and volcanic soils of the Phillipines. It will probably not be strong enough for really hard or stoney soils. The plan is to loan this to IRRI if suitable equipment is not already available there.

Residue analysis: Samples from the experiments involving agent 'orange' should be analysed for 2,4,5-T and those from the experiment with 'white' for picloram. If possible 2,4-D should be analysed in samples from both experiments but this should be regarded as of lower priority since 2,4-D is known to degrade more rapidly in soil than 2,4,5-T. At the time of writing (22nd November) it is not known for certain which laboratories will carry out the analyses. Dr. Chandler has offerred to explore the possibility of the work being undertaken by IRRI and Professor Ho and Dr. Lang will investigate facilities in Saigon. In either case, communication should be established with Dr. R. J. Hance of WRO who has experience of the techniques and problems involved and who, if necessary, is prepared to make a short advisory visit to the laboratories concerned.

Supplementary studies: In addition to the basic experiments proposed above, it would be valuable if the work could be extended to include (i) plots of mangrove forest in a natural state sprayed by aircraft with 'orange' and 'white' at the standard rate of

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* It follows from this that 50 residue determinations should be made immediately after mixing.

3 gal/ac (Vietnam only); (ii) some bioassays of residual phytotoxicity, in particular the planting of mangrove seedlings in situ; (iii) measurement of rate of disappearance when 'orange' and 'white' are applied at lower doses representing drift levels or a dose likely to reach the soil direct under a forest canopy (only if the aircraft applications described below prove not to be feasible or if assessment of the hand-sprayed plots 1 and 6 weeks after treatment indicate a slow rate of dissipation).

(i) Aircraft-sprayed plots. The size and replication of the plots should be decided by the ecology group but it seems likely that two unreplicated strips each of 'orange' and 'white' (at 3 gal/ac) will be all that is feasible with a buffer untreated strip between. As already noted, the route of entry of the herbicide into the soil is likely to be largely via the leaves and twigs of the treated forest and if possible an estimate of the herbicide dose reaching the soil directly or indirectly should be obtained by (i) use of an oil-soluble dye such as Waxoline Red in the spray solution, placing strips of filter paper in suitable holders a few inches above the soil surface prior to spraying, eluting the dye from the filter papers after spraying and assessing dosage by colorimetry; (ii) collecting samples of leaves falling from the trees at intervals after spraying and estimating the herbicide reaching the soil by this route. In either case variation in deposit from place to place within a sprayed plot is likely to be extremely large and unless a considerable number of random samples can be taken and analysed, the chance of any reliable data resulting will be small. Little guidance can, unfortunately, be given on the minimum number of samples required to produce a reasonably low coefficient of variation and it seems that at the best only a rough estimate of herbicide levels reaching the soil can be obtained. For the initial sampling of spray deposit, a reasonable procedure might consist of five rows of 10 samples at right angles to the direction of spraying, spaced at regular intervals along the swathe. Two-inch wide strips of filter paper have proved satisfactory for this purpose, being kept flat in light metal holders which can easily be made up from thin gauge sheet aluminium. If each plot consists of two spray swathes, soil samples should not be taken from the vicinity of the junction of the adjacent swathes, since the dose is likely either to be excessive or much lower than average according to exact position of the aircraft on each run and prevailing met conditions. In Vietnam, theprocedure adopted for soil sampling for residue analysis of the aircraft-sprayed plots will doubtless be more a question of feasibility rather than of statistical requirement. If possible cores should be analysed separately as above. If not, bulking and sub-sampling can be done providing thorough mixing can be achieved (probably necessary to do this by hand) and there is only a short interval between mixing and analysis - or deep-freeze storage. Again 20 cores per plot should, if possible, be regarded as a minimum with a selection of sites representative of different densities of canopy. Since the total dose reaching the soil is likely to be considerably less than in the cleared plots and the period of dosage will cover days, if not weeks, following

Dates of sampling

treatment according to the rate of leaf fall, more frequent samplings will be required, particularly in the early stages, e.g. 0, 1, 2, 4 and 8 weeks after spraying. If it is decided to collect leaf samples for herbicide analysis, wicker or woven grass baskets of local manufacture should be considered. They would be cheap, light, inconspicuous and easily secured above high tide level to 3 canes.

(ii) Bioassays. In addition to the chemical residue determinations outlined above, it would be valuable if simple bioassays could be undertaken to provide a qualitative assessment of the disappearance of phytotoxic residues. Considerable experience of field and laboratory techniques for bioassay herbicide residues in soil is available at WRO but modification would be required for mangrove soils. For the latter the most realistic approach would seem to be sequential plantings of mangrove seedlings direct into the cleared hand-sprayed plots and the aircraft-sprayed strips.

(iii) Lower doses on cleared plots. A dose of about $\frac{1}{3}$ gal/ac of either agent ('orange' or 'white') might be appropriate, but should be adjusted according to the disappearance rate indicated by the early assessments in the hand-sprayed plots. This should not receive high priority, unless the latter indicate that little loss of herbicide has occurred or if in the case of mangrove soils it should not prove possible to undertake the aerial spraying trial.

II FOREST SOILS

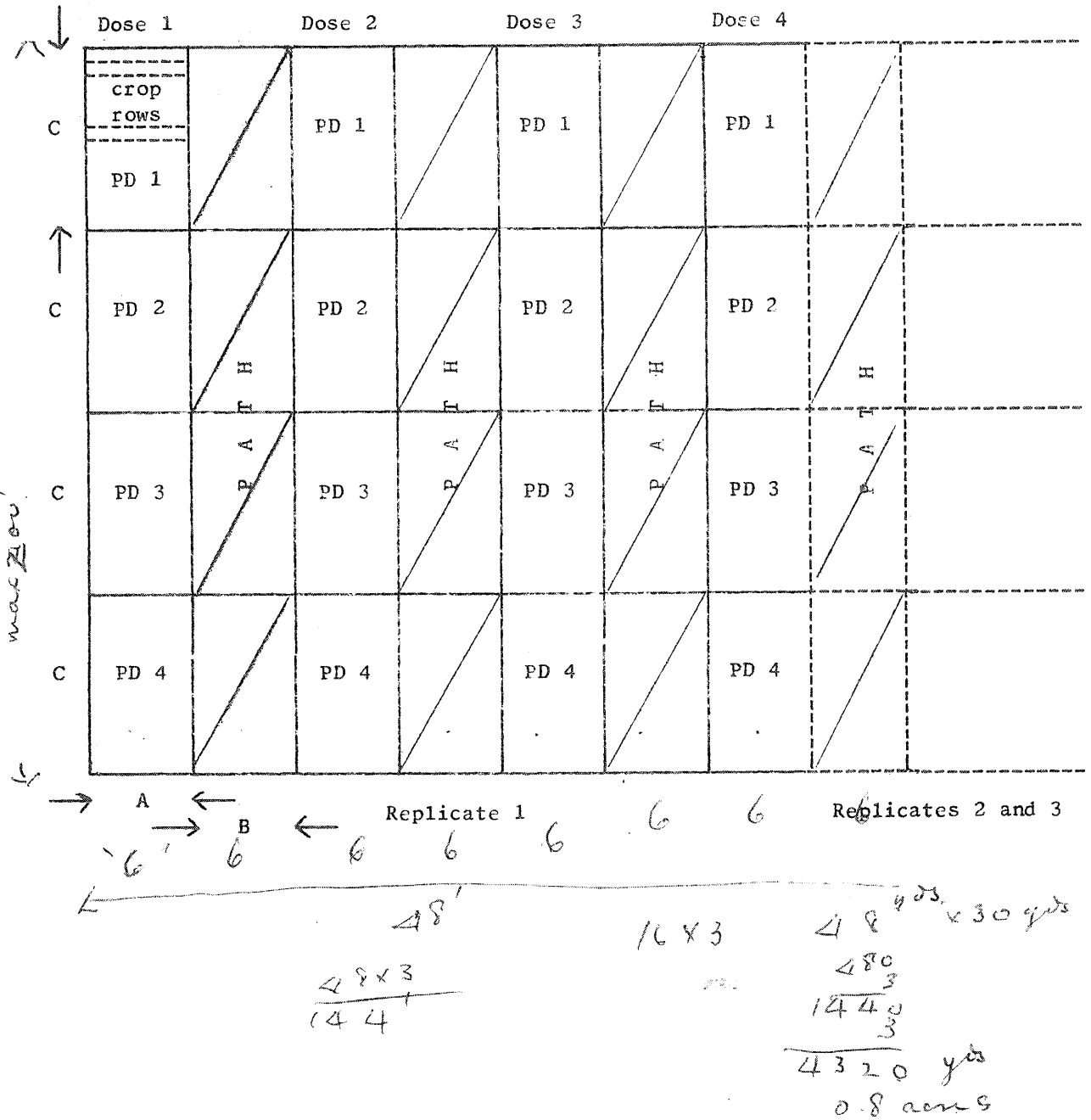
The general programme described above is also relevant to forest soils but will of course require modification according to local conditions. In the Phillipines Dr. Chandler has reported that it is likely to be possible to clear the undergrowth away to lay down the main experiments in a standing forest. In Vietnam a rubber plantation is likely to be the most suitable if a site, security and co-operation can be obtained. In both forest and mangrove there is some risk of damage to the trees, particularly from agent 'white' and agreement about this will need to be reached with the collaborator.

III AGRICULTURAL STUDIES

To obtain background knowledge of the persistency of residual effects in the soil arising from drift or the accidental application of the 'orange' and 'white' formulations to arable crops the following experimental layout is proposed (see diagram on page 7), but with separate experiments for the two formulations.

There are advantages of ease of application in having long relatively narrow plots which should be sprayed at rates of 0.0, 0.3, 1.0 and 3.0 gallons of the agent per acre. As stated earlier each formulation should be diluted to give a cover of 30 gal/ac.

SUGGESTED LAYOUT FOR AGRICULTURAL STUDY



A - plot width determined by swathe width of sprayer butnot less than 6 ft

B - path width not less than 6 ft (unless bunds between plots)

C - 50 ft (40 ft in rice excluded)

PD - Planting date

NOTE: Position of planting dates and doses should be randomised

At four intervals after spraying rows of seed in the case of corn (maize), sorghum, groundnut and mung beans should be sown transversally across the control and treated plots, together with cuttings of sweet potato and transplants of rice - see later. At each sowing there should be a minimum of 4 rows with records of plant establishment and growth made on the two middle rows. These observations should be restricted to the early vegetative phase - say 4-6 weeks from sowing or planting - when any abnormalities in the pattern of growth should be noted, together with final counts made at the end of the 4-6 weeks of leaf and stem or tiller number and maximum height, as minimum requirements.

After making the observations all the plants should be removed so that there will be no interference with adjacent younger plants that may have been sown at a later date.

The layout of the plots will be dependant on whether during the life of the experiment any dry period is likely to be so pronounced that it will not permit of the successful establishment and subsequent growth at each of the "sowing" dates. If drought is not a hazard, the ground is level and there is little risk of surface wash in heavy rain the plot unit should be one where the length is sufficient to accommodate the four sowing dates of all the selected crops. But, clearly these conditions will exclude paddy rice with its requirement of standing water. The simplest arrangement would be for a separate rice experiment with bunded plots.

Assuming that the distance between rows will be about 1.5 feet for corn, sorghum, groundnut and mung bean and 3.0 feet for sweet potatoes the total plot length will be 200 feet (50 x 4) which would allow narrow gaps between sowing dates. If this is an awkward shape the plot could be split into 2 parallel 100 ft lengths, each with 2 sowing dates.

The width of the plots should be related to the effective width of the sprayboom and for uniformity of application plot width should be in multiples of boom width, with a minimum of 6 feet.

This split plot design is aimed at minimizing possible contamination between doses and so preferably the width of the paths separating the unit plots, compounded of dose and time of sowing, should be relatively wide - about 6 feet. Again, because of the greater mobility of the 'white' formulation the two experiments should be well separated.

If there is a danger of water stress and all the plots have to be watered from time to time the layout should be substantially the same but the details will be dependant on the method of irrigation.

The intervals between sowing dates will be determined by (i) when the experiment is started and (ii) the completion of the observations following the last sowing date. If as planned the experiments are initiated in January at the IRRI and in January or February at Mytho and all the data to be are/ready by the autumn for incorporation into the final report, the intervals between sowing dates on a logarithmic scale should be 3, 6, 12 and 24 weeks from the time of spraying.

Without a knowledge of local conditions only a guestimate of the land requirement can be made. Allowing for a three-fold replication about an acre should suffice.

The plots should be hand-weeded as necessary to ensure that weeds do not interfere with i) growth of the crops ii) seedbed preparation and crop establishment.

Appendix

LIST OF EQUIPMENT LIKELY TO BE NEEDED

MARKING OUT PLOTS

Pegs

Labels

String

Tape measure*

Mallet or hammer

SPRAYING

Sprayer*)
Sprayboom*) pre-calibrated

4 plastic containers of 1.5 US gal capacity for mixing up spray solution

Clean water 20 gal in clean containers, e.g. petrol or jerry cans

Measuring jug 1 US gal

" " 1 pint

Stopwatch*

Plastic funnel with fine mesh filter or funnel with muslin for filtering

Rubber gloves

Light rubber or canvas-rubber boots

AERIAL APPLICATIONS

Dye

Filter paper strips*

Aluminium holders*

Baskets for collecting leaves

Canes for supporting basket clear of water

SAMPLING

Corer* (a new corer is being constructed at WRO and will be available for use in Philippines in addition to the one already in Saigon)

Double polyethylene bags* (500)
or
Metal containers (already in Saigon)
or
Plastic jars with screw tops

Plastic labels*

Pens with waterproof ink for marking*

NOTE: Items marked with * will be provided by WRO

National Academy of Sciences
Committee on effects of herbicides in Vietnam

PERSISTENCE OF HERBICIDES 'ORANGE' AND 'WHITE' IN A FOREST SOIL

Preliminary record of an experiment at Los Banos, Philippines

OBJECT

To determine the rate of disappearance of 2,4,5-T, 2,4-D and picloram applied as defoliation agents 'Orange' and 'White' on a forest soil during the dry season.

TREATMENTS

(i) Agent 'Orange' a 50/50 mixture of technical grade n-butyl esters of 2,4,5-T and 2,4-D applied at 2.7 gal (US) product in 30 gal (US) diluent/ac equivalent to 11.7 lb 2,4,5-T a.e. + 11.4 lb 2,4-D a.e./ac. The 'Orange' was formulated as an emulsifiable concentrate (e.c.) and then diluted with water.

(ii) Agent 'White' (Dow Chemical product Tordon 101) containing 2.0 lb 2,4-D a.e./gal (US) and 0.54 lb picloram a.e./gal (US) both as the tri-isopropanolamine salt applied at 2.8 gal (US) product in 32 gal/ac (US) equivalent to 5.6 lb 2,4-D (a.e.) + 1.5 lb picloram (a.e.)/ac. The product is an aqueous concentrate and was diluted with water for spraying.

'Orange' was formulated as an emulsifiable concentrate using an adjuvant consisting of 60 parts Atlox 3404, 90 parts Atlox 1186 and 850 parts kerosene by weight. The concentrate was prepared by mixing one part of the adjuvant with two parts 'Orange' by volume. On adding water a satisfactory emulsion was immediately formed with gentle stirring. In the laboratory test some settling out of oily components occurred during overnight storage, but these disappeared readily on stirring. For spraying a knapsack sprayer was partly filled with water to which the 'Orange' e.c. was added and mixed. The remaining water was then poured into the sprayer which was thoroughly shaken to ensure complete dispersion. The same procedure was used for diluting 'White'.

Approximately half the total dose of each product was applied during the evening of 18th January 1972 (overcast, cool); the remainder the following morning (sunny, warm). The time taken to spray each plot was measured by stop-watch to allow the normal dosage to be adjusted to take

into account variations in speed of walking caused by the difficult terrain. The doses given above are those calculated to have been actually applied.

LAYOUT AND SITE

One plot 15 x 30 m was treated with 'Orange' and an adjacent plot of the same size treated with 'White'. Plots were unreplicated due to site restrictions. For spraying each plot was marked out into nine 2-yd strips, the width of the spray swath. The site near Los Banos kindly provided by the University of the Philippines, College of Agriculture, was located in a clearing in the forest reserve. The clearing, which did not exceed 1 ha has probably been subjected to shifting cultivation and the vegetation was now dominated by grasses and herbs with a few bushes and bananas. There was a heavy litter layer overlying a heavy tenacious red clay soil. The plots were situated on a 20^{o} slope facing south east.

MANAGEMENT

Before spraying, two thirds (10 x 30 m) of each plot was cleared of vegetation to expose the litter layer. On the remaining third the vegetation was slashed and left on the plot providing a dense cover of wilting freshly cut trash up to 20 cm deep. After spraying the plots will be left to regenerate and no further management is proposed.

SAMPLING

Before spraying, 5 soil cores were taken from each plot to 25 cm depth to provide blanks for the chemical analysis of residues. In addition a pit 50 cm deep was dug in each plot and a core extracted from the 50 - 75 cm soil layer. The internal diameter of the corer orifice was 4.6 cm.

After spraying, during the afternoon of 19th January, 5 cores to 25 cm depth were taken at random in each quarter of the cleared part of each plot, i.e. 20 cores per plot. Each core was placed in a plastic bag, stored in a refrigerator within 4 hours ($0^{o}C$) and the arrangements made that they should be moved to a 'deep freeze' within 36 hours. Subsequent sampling is intended at approximately 3, 9 and 27 weeks after spraying. At each date 20 cores should be taken as above in each plot, but the core depth should be increased to 75 cm, (underlying rock permitting) each core being sub-divided into 3 x 25 cm sections. If the tenacious nature of the soil does not permit using the corer to this

depth, the deeper sampling should be undertaken by extracting samples from the face of dug pits.

RESIDUE ANALYSES

Levels of each herbicide in each core section will be estimated by Dr. T. Yoshida, Microbiology Section, IRRI, in consultation with Dr. R. J. Hance of WRO, by appropriate chemical methods. There may be a problem of expressing residues in terms of absolute levels in each column, since core samples varied greatly in weight on account of variable bulk density.

SPRAY DEPOSIT ANALYSES

During each spray operation 10 Whatman chromatographic paper strips (45.7 x 7.6 cm) each with a receiving area of 268.3 cm^2 were placed at random in each plot. The sprayed papers were collected up within a few minutes of herbicide application, folded and rolled and those from each plot were stored together in separate double plastic bags. These were transferred to a refrigerator within a few hours and hopefully to a deep freeze within 24 hours. The samples from each plot for each application should be extracted and analysed for deposit level of the respective herbicides.

J. D. FRYER

January 1972

CODE FOR LABELLING SAMPLES

'Orange'		'White'	
5 samples (16-20) (d)		5 samples (16-20) (d)	
5 samples (11-15) (c)		5 samples (11-15) (c)	
5 samples (6-10) (b)		5 samples (6-10) (b)	
5 samples (1-5) (a)		5 samples (1-5) (a)	

(Shaded areas are those on which vegetation cut but not removed)

CODE:

LB - Site Los Banos

C - Control (pre-spraying samples)

TO - Treated 'Orange'

TW - Treated 'White'

(a-d) equal quarters a = bottom d = top of plot

i.e. LBTO 1 (a) equivalent to Los Banos Treated 'Orange' 1 (a) (first sample of sub-plot a)