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# EFFECTS OF ULTRAVIOLET RADIATION ON PLANT VIRUSES AND ON THE CAPACITY OF HOST PLANTS TO SUPPORT THEIR MULTIPLICATION

BY

A. KLECZKOWSKI

That ultraviolet radiation (UV) can kill plant cells was first recognised by Maquenne & Demoussy in 1909, and that it inactivates a plant virus, namely tobacco mosaic virus, was first demonstrated independently by Mulvania and by Smith in 1926. The emphasis in early research was in comparing the rates of virus inactivation with the rates of killing bacteria, which it was hoped would shed light on the nature of viruses. Mulvania (1926) and Smith (1926) concluded that, as tobacco mosaic virus is much more resistant to UV than are bacteria, it is more comparable to an enzyme than to a bacterium. They compared their results with the virus directly with those obtained by other workers with bacteria, regardless of possible differences in intensities of irradiation or of the fact that the results were obtained in environments that differed widely in their capacities to absorb UV. No significance can, therefore, be attached to the comparisons. This was recognised by Duggar & Hollaender (1934a, b), who made comparisons by irradiating tobacco mosaic virus and different bacteria simultaneously in the same medium. They also found that the virus is much more resistant to UV than all the bacterial species that they tested. However, the considerable variation in susceptibility to UV between bacterial species and even between different stages of growth within one species (Zelle, 1955), and also between different plant viruses (Bawden & Kleczkowski, 1955), make a complete generalisation impossible.

The early trends in research on inactivation of plant viruses by UV very soon assumed the modern character when such problems as action spectra, kinetics, quantum yields, etc., were investigated. By contrast, research on the lethal effect of UV on plant cells progressed little and has not reached a quantitative stage. A new aspect of the subject was opened with the discovery of the phenomenon of photoreactivation, that is that some of the effects of UV on both plants and viruses can be reversed by visible light.

## *Action spectra*

Tobacco mosaic virus is the only plant virus whose action spectrum for inactivation by UV has been determined, first by Duggar & Hollaender (1934a, b) and then slightly corrected (Hollaender & Duggar, 1936). The spectrum of the type strain of the virus is

rather unusual. Plotting relative efficiency of radiation against wavelength gave no peaks. The line rose steadily as the wavelength decreased from 290  $m\mu$ , slowly at first and then rapidly below 250  $m\mu$ . This has been confirmed recently by Siegel & Norman (1958) and by Rushizky, Knight & McLaren (1960). The action spectrum does not resemble the absorption spectrum of nucleic acid, of virus protein or of the whole virus. The action spectrum of the strain U2 differs from that of the type strain in that, instead of the rise below 250  $m\mu$ , it shows a slight drop; it thus has a peak in the vicinity of 260  $m\mu$ , and so slightly resembles the absorption spectrum of nucleic acid.

Preparations of the infective nucleic acid from tobacco mosaic virus behave very differently, for Rushizky *et al.* (1960) found that the quantum yields for the inactivation were independent of wavelength, both with and without photoreactivation, so that the action spectrum closely resembles the absorption spectrum of nucleic acid. They also found that reconstituted virus, i.e., the product of recombining separated nucleic acid and protein, behaves like the original virus.

Assuming that infectivity of the virus is a function of its nucleic acid component, the action spectrum of the nucleic acid is therefore drastically modified by the protein component either in the original or in reconstituted virus. This may be because the bonding between protein and nucleic acid protects the nucleic acid from damage by UV (Siegel, Wildman & Ginosa, 1956; McLaren & Takahashi, 1957; Bawden & Kleczkowski, 1959), and the degree of protection may depend on the wavelength. Some kinds of damage by UV in the protein may also interfere with initiation of infection, and such damage may occur predominantly within a particular range of wavelengths. The fact that the action spectrum of the U2 strain of tobacco mosaic virus deviates from that of the isolated nucleic acid less than does that of the type strain, and the fact that nucleic acid in strain U2 is much less protected from damage by UV than is nucleic acid in the "type" strain, suggests that the protection may contribute to the deviation of the action spectrum of the whole virus from that of the isolated nucleic acid.

#### *Kinetics of inactivation*

Gowen & Price (1937) and Lea & Smith (1940) concluded that inactivation by UV of tobacco mosaic virus, tomato bushy stunt virus and a tobacco necrosis virus proceeds according to the first-order kinetics, i.e., plotting logarithms of proportions of residual infectivity against doses of irradiation gives straight lines. These authors assumed that residual infectivity over wide ranges is exactly proportional to the numbers of lesions produced on leaves inoculated with irradiated preparations, which is not true in general. Nevertheless, Oster & McLaren (1950), who computed the extent of inactivation by finding dilutions at which irradiated and control solutions of tobacco mosaic virus gave equal numbers of lesions, and Bawden & Kleczkowski (1953), who obtained residual infectivities of irradiated preparations of tobacco mosaic virus, tomato bushy stunt virus and a tobacco necrosis virus, by interpolation from dilution curves obtained by inoculating control virus preparations over a

range of dilutions, also found that inactivation proceeds, approximately at least, according to the first-order kinetics.

Because of the first-order kinetics, most workers concluded that virus particles are inactivated by "single hits", i.e., by single quanta of radiation energy that happen to "hit" regions essential for infectivity. That this may, but need not, be so was pointed out and discussed by Kleczkowski (1960). One fact that throws doubt on the "single-hit" hypothesis, without, however, disproving it, is the extreme smallness of quantum yields. For example, a particle of tobacco mosaic virus absorbs on the average about 25,000 quanta of the radiation of 254 m $\mu$  before it is inactivated (Oster & McLaren, 1950; Kleczkowski, 1954).

#### *Photoreactivation*

The phenomenon called photoreactivation was discovered by Kelner in 1949, when he found that the proportion of *Streptomyces griseus* conidia that survived UV irradiation was greater when they were subsequently exposed to visible light than when kept in darkness. That the phenomenon extends to some plant viruses, and to leaf cells of such plants as French bean, was found by Bawden & Kleczkowski (1952, 1953). Irradiated viruses do not have their infectivity increased by exposure to visible light *in vitro*. The phenomenon operates through some light-sensitive mechanism in the host cell and shows by the proportion of surviving infective virus being greater when plants are exposed to daylight after inoculation than when they are kept in darkness. Keeping the plants in light or darkness for a period of time before inoculating them with UV-irradiated virus does not affect the apparent proportions of surviving infectivity of the virus.

Whether exposure to visible light reverses the damage caused by UV to plant viruses and to leaf cells, or counteracts the damage in some other way, has yet to be established. However, Lennox, Luria & Benzer (1954), by studying the rates of repeated inactivation and photoreactivation of a bacteriophage inside its host cell, showed that the change caused by UV in the bacteriophages is probably reversed by photoreactivation, and this conclusion probably applies generally.

Photoreactivation increases the residual infectivity of UV-irradiated virus preparations, but does not restore it to its original level. Hence the radiation causes two kinds of damage, only one of which is photoreversible. Kleczkowski (1960) gave evidence that the reversible and irreversible damage occur independently and that the irreversible damage is not a further change in particles already changed reversibly.

The inactivation of plant viruses by UV apparently proceeds according to the first-order kinetics, whether or not photoreactivation operates. Thus, if  $v$  is the dose of irradiation, the proportion of residual infectivity with photoreactivation is  $p_{\text{light}} = \exp(-k_{\text{light}}v)$  and without photoreactivation  $p_{\text{dark}} = \exp(-k_{\text{dark}}v)$ , where  $k_{\text{light}}$  and  $k_{\text{dark}}$  are constants characteristic for a given virus in a given set of conditions. A ratio  $k_{\text{light}}/k_{\text{dark}}$  equal to one means there is no photoreactivation, and any excess of the ratio over one shows the extent of photoreactivation. The ratio differs considerably with

different viruses (Bawden & Kleczkowski, 1955). It may possibly differ even with one virus, depending perhaps on the species of host plant in which photoreactivation is obtained, or on the condition of the host plant, but this has yet to be investigated.

Of several plant viruses now tested, all have shown the phenomenon of photoreactivation except tobacco mosaic and tobacco rattle viruses. Although these two are not photoreactivated when irradiated intact, photoreactivation is shown when their freed nucleic acids are irradiated. (Bawden & Kleczkowski, 1959; Harrison & Nixon, 1959.)

A fact immediately obvious from comparing the rates at which intact tobacco mosaic virus and its free nucleic acid are inactivated by UV is that the nucleic acid is much more resistant to inactivation when it is a part of intact virus than when free (Siegel, Wildman & Ginoza, 1956; McLaren & Takahashi, 1957; Bawden & Kleczkowski, 1959). When the isolated nucleic acid is irradiated, about half of the absorbed radiation energy seems to be concerned with the kind of damage that is reversible by photoreactivation and the other half with the irreversible damage. When intact virus is irradiated, inactivation by radiation energy absorbed per unit of nucleic acid progresses at a rate that is roughly only about one-tenth of that of free nucleic acid, and no photoreactivable damage occurs (Bawden & Kleczkowski, 1959).

The probable reason for the nucleic acid being more resistant to UV when inside the virus than when free is that the type of bonding between the nucleic acid and the protein reinforces the structure of components of nucleic acid. The degree to which nucleic acid is protected by the protein differs with different viruses, and even with different strains of the same virus, as for example with strains U1 and U2 of tobacco mosaic virus (Siegel, Wildman & Ginoza, 1956). Results obtained by Kassanis (1960) suggest that the nucleic acid of a tobacco necrosis virus may be protected only very little or not at all by the protein component. These differences can be explained by assuming differences in the nature of bonding between protein and nucleic acid.

The lack of photoreactivation with tobacco mosaic virus when irradiated intact could have two explanations. The bonding with protein may protect the nucleic acid from the photoreversible kind of damage, while allowing the irreversible kind, or it may prevent visible light from reversing changes caused by UV radiation. That the first is the correct explanation was shown by the failure to obtain photoreactivation when plants were inoculated with the nucleic acid isolated from virus irradiated while intact (Bawden & Kleczkowski, 1959).

Of the plant viruses yet tested, potato virus X showed the phenomenon of photoreactivation most strongly, and using this virus, photoreactivation could be roughly timed. With tobacco plants inoculated with UV-irradiated potato virus X and kept at about 20°, it mattered little whether they were in light or in darkness during the first 30 minutes. After that period had passed, most photoreactivable virus was photoreactivated during about 15 minutes in ordinary daylight, but only when the plants were exposed to light during the next hour. Thus the condition of the virus particles

that are reversibly inactivated by UV changes twice in inoculated leaves. The first change makes them ready for photoreactivation, and, if photoreactivation does not then occur soon, the second change makes them inactive irreversibly (Bawden & Kleczkowski, 1955).

The irradiated free nucleic acid from tobacco mosaic virus behaves differently. Most reversibly inactivated nucleic acid seems to become photoreactivable either immediately or within a few minutes after inoculation to the host plant (*Nicotiana glutinosa*); if it is not photoreactivated within an hour or so, most of it becomes irreversibly inactive (Bawden & Kleczkowski, 1960).

The lethal effect of UV on cells of higher plants can also be reversed by photoreactivation (Bawden & Kleczkowski, 1952; Tanada & Hendricks, 1953; Benda, 1955; Chessin, 1958), but there is no information about the rate at which cells are killed by UV radiation, the extent to which this can be reversed by photoreactivation, the rate of photoreactivation and the effect of time-interval between exposure to UV and to visible light.

Action spectra for photoreactivation of UV-effects on infectivity of plant viruses or on viability of plant cells have not yet been obtained. However, by the use of selective light filters Tanada & Hendricks (1953) found that the lethal effect of UV on cells of leaves of soybean was prevented by light of wavelengths shorter than 450 m $\mu$ , and Chessin (1958) found the same with French bean leaves and also with potato virus X. These results fit with those previously obtained with other materials, such as a bacteriophage (Dulbecco, 1950), *Escherichia coli* and *Streptomyces griseus* (Kelner, 1951). The action spectra for photoreactivation of these materials have peaks near 350 or 450 m $\mu$  and fall to zero below 300 and above 500 m $\mu$ .

#### *Loss of infectivity and structural alteration in virus*

The photochemistry of inactivation of plant viruses by UV is still unexplored. All the information shows that irradiation destroys infectivity without causing any gross changes in the structure of the particle. That virus preparations could be inactivated but still retain their ability to crystallise and to react with specific antisera has long been known (Stanley, 1936; Bawden & Pirie, 1938a, b). Oster & McLaren (1950) found that tobacco mosaic virus preparations that had lost more than 98% of their infectivity showed no change in viscosity, sedimentation constant, optical turbidity, isoelectric point, appearance in the electron microscope or UV absorption spectrum. McLaren & Takahashi (1957) also found that infective nucleic acid isolated from tobacco mosaic virus did not alter appreciably either in viscosity or in UV absorption spectrum after it had lost 99.99% of infectivity. All this suggests that neither the protein nor the nucleic acid components are much altered when infectivity is lost.

The structural changes responsible for loss of infectivity are unknown, but as pyrimidines are very much more susceptible than purines to changes by UV, it is reasonable to suspect that loss of infectivity results from damage in pyrimidine residues of the virus nucleic acid. Whether the photoreversible change caused by UV in virus nucleic acid is the reversible hydrolysis in the double bond between 5 and 6 positions in cytosine and uracil, as suggested by

Shugar & Wierzchowski (1958), is still questionable, for it has so far been reversed only by acid, alkali or heat, and whether in suitable conditions it can also be reversed by exposure to visible light still remains to be tested.

*Inactivation of the capacity of plants to support virus multiplication*

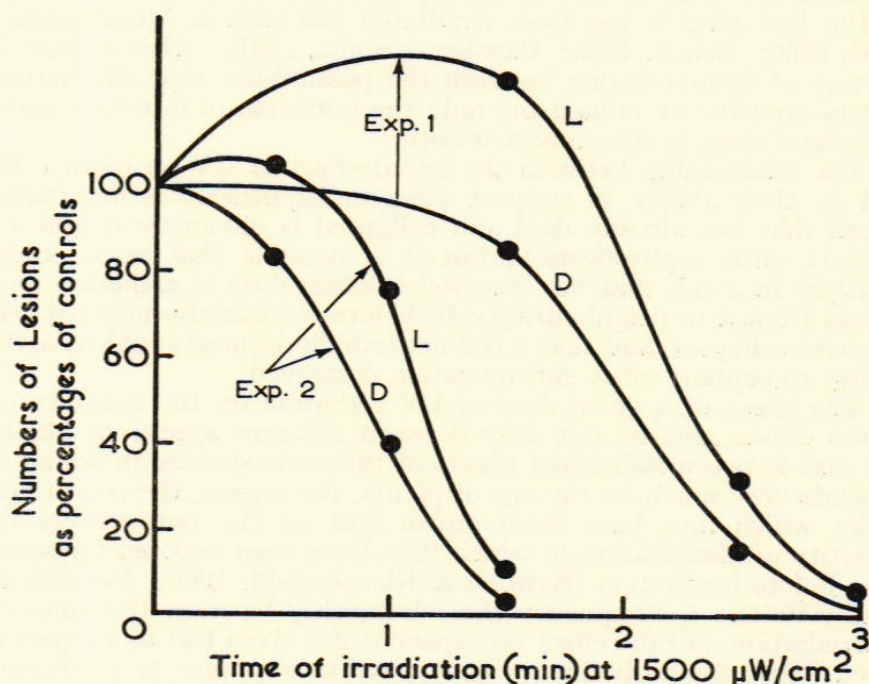
The word "capacity" is used here to mean the ability of a leaf to support multiplication of a virus to the extent of forming local lesions. The only current method of measuring the effect on capacity of exposing a leaf to UV radiation is to observe the effect on numbers of lesions formed by virus preparations that are inoculated to the leaf after it has been irradiated (Bawden & Kleczkowski, 1952, 1960; Benda, 1955; Bawden & Sinha, 1961). Hence there is no way of differentiating between the possibilities that irradiation affects capacity by influencing only the initiation of infection, only some later stage in the process or both.

The relationship between the lethal effect of UV on plant cells and on their ability to support virus multiplication is uncertain. A cell that has already died and collapsed is obviously unable to support virus multiplication, but it is possible that virus could multiply in a cell that has received a lethal dose of radiation and spread from it to neighbouring cells before the initially infected cell dies and collapses, and that a cell not lethally injured could have its ability to support virus multiplication destroyed.

The effect of a given dose of UV radiation on the capacity of leaves differs greatly, not only between different species of plants but also between individual plants of the same species, in which it depends very much on the age of plants, the season, the conditions under which they have been grown and on the temperature or quantity of illumination to which they have been exposed for some time before irradiation (Bawden & Kleczkowski, 1960; Bawden & Sinha, 1961). Consequently the relationship between the amount of irradiation and the effect on capacity of a given leaf to support a given virus is an individual property, and can no more be expressed in generally applicable terms than can the relationship between virus concentration in the inoculum and the number of lesions it will produce. The conditions so far known to increase susceptibility of capacity to UV also increase susceptibility to virus infection, but whether the connection is more than fortuitous has yet to be established.

The total effect of UV radiation on capacity can be determined only by putting leaves in darkness after they are irradiated, for exposure to daylight counteracts the damage (photoreactivation) (Bawden & Kleczkowski, 1952, 1960; Benda, 1955; Bawden & Sinha, 1961). The figure shows the results of two experiments in which *Nicotiana glutinosa* leaves were exposed to different doses of UV radiation immediately before they were inoculated with tobacco mosaic virus, after which half of the leaves were kept for 24 hours in darkness and half were exposed to daylight. The much greater susceptibility to UV of the leaves used in Experiment 2 is obvious, but the effect of photoreactivation is more spectacular in Experiment 1. In both experiments the numbers of lesions formed after photoreactivation depended on the dose of UV irradiation. With

small doses, photoreactivation increased the numbers above that of the non-irradiated controls; with intermediate doses, it restored them to the levels of the controls; with larger doses it increased the numbers but did not restore them to the levels of the controls. The effects of irradiation are obviously complex. Some are reversed by photoreactivation, and some are not. The increase in numbers of lesions above the original level when leaves were exposed to daylight after exposure to small doses of UV radiation may mean that the radiation can increase the leaf's capacity directly, or that photoreactivation can over-compensate the radiation damage and thereby



The figure shows results of two experiments in which halves of leaves of *Nicotiana glutinosa* were irradiated at  $\lambda$  254  $m\mu$  and then the whole leaves were inoculated with a solution of purified tobacco mosaic virus; L = leaves in daylight after inoculation; D = leaves in darkness for 24 hours after inoculation.

make conditions in some cells such that infection can now occur, although it would not had the cells remained in their original state.

The time required for photoreactivation to be completed after leaves have been given different doses of UV radiation has not been studied in detail, but with moderate doses it probably happens in a few hours in ordinary daylight. The results of inoculating leaves immediately after they have been irradiated differ with different viruses and with different types of inocula. The figure shows that with tobacco mosaic virus considerably more infections are obtained when irradiated leaves are exposed to daylight than when kept in darkness. However, this does not happen with inoculum of free nucleic acid from tobacco mosaic virus, with which no more, or only very slightly more, lesions are obtained on UV-irradiated leaves



exposed to daylight than on those kept in darkness (Bawden & Kleczkowski, 1960). When the irradiated leaves are exposed to daylight for 3 hours before they are inoculated the nucleic acid behaves like the intact virus, and the numbers of lesions formed by the two types of inoculum are equally increased by exposure to daylight. It seems that, whereas the nucleic acid cannot survive infective in irradiated cells until their capacity is restored by photoreactivation, intact particles of the virus can. Different viruses differ in their ability to survive in irradiated cells undergoing photoreactivation. The Rothamsted tobacco necrosis virus inoculated to irradiated French bean leaves behaves similarly to the nucleic acid from tobacco mosaic virus (Bawden & Kleczkowski, 1952), whereas red clover mottle virus is intermediate in its behaviour between tobacco mosaic virus and the tobacco necrosis virus (Bawden & Sinha, 1961). The nucleic acid of this virus also seems more stable *in vivo* than that of tobacco mosaic virus, for when inoculated to leaves immediately after they are irradiated it gives more lesions on leaves kept in the light than in the dark, though the effect of the light is smaller than with inocula of intact virus particles.

#### *Irradiating virus-infected leaves*

Arthur & Newell (1929) found that tobacco mosaic virus "could be killed with a short exposure (to UV-radiation) when spread upon the plant leaf surface if irradiated at once. If irradiated the day following inoculation there was no appreciable killing of the virus. It is apparently impossible to inactivate the virus when it has penetrated far into plant tissue, although irradiations were given of sufficient intensity and quality to kill the whole upper surface of plant leaves".

The subject of the effect on viruses of irradiating virus-infected plants remained (to the reviewer's knowledge) untouched for 25 years, until the results of irradiation studies with bacteria infected with bacteriophages (Luria & Latarjet, 1947; Benzer, 1952; Benzer & Jacob, 1953) stimulated further work (Bawden & Harrison, 1955; Siegel & Wildman, 1956). Unfortunately some concepts brought across from the work with bacteriophage were inapplicable to infected leaves and have led to conclusions that further work has shown to be unjustified. The effect of irradiation has been assessed by comparing the numbers of lesions that develop on irradiated halves of leaves with those that develop on unirradiated halves. Differences were attributed solely to the inactivating effect of radiation on virus particles, whereas what was measured was the effect on what can be called "infective centres", and their exact nature is unknown. They may be virus-infected cells or groups of cells, virus particles that are about to infect or cells that are about to be infected. Thus, destroying an infective centre may mean inactivating virus, or affecting cells or virus-cell association.

Effects of irradiating leaves at different times after inoculation on numbers of lesions not only confirmed Arthur & Newell's (1929) conclusion that virus soon spreads from epidermis into deeper tissue where it is protected from the radiation but has also established some other phenomena. Thus, with a tobacco necrosis virus in French bean leaves (Bawden & Harrison, 1955), and with tobacco mosaic

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virus in leaves of *Nicotiana glutinosa* (Siegel & Wildman, 1956), the resistance of infective centres to UV radiation remains unchanged for a time after inoculation, after which it increases steadily and rapidly, until ultimately doses of the radiation much larger than those initially required to prevent lesion formation were ineffective. The reason for the lag period after inoculation before infective centres start to increase their resistance to irradiation (which differs characteristically with different viruses and virus strains) remained without any explanation until Siegel, Ginoso & Wildman (1957) found that it was abolished when plants were inoculated with free nucleic acid isolated from tobacco mosaic virus instead of with the intact virus. This has since been found to be so also with a tobacco necrosis virus (Kassanis, 1960) and with red clover mottle virus (Bawden & Sinha, 1961). The difference between the behaviour of the nucleic acids and intact viruses suggests that the nucleic acids dispense with some early step in the infection process required by intact viruses, and this perhaps provides the strongest evidence for the current idea that a first step in infection normally entails the nucleic acid moiety separating from the protein moiety of the virus.

This idea may be correct, and the results of some other experiments fit readily to it, as, for example, the fact that irradiated nucleic acid from tobacco mosaic virus is photoreactivable immediately it is inoculated to leaves, whereas particles of potato virus X have to wait for 30 minutes or more. However, not all experimental results fit to the idea. For example, as tobacco mosaic virus survives in irradiated leaves through the period while the leaves are photoreactivated and its nucleic acid does not, and as nucleic acid is much more susceptible to UV radiation than the intact virus, if the lag period is the time required for the nucleic acid to become free, irradiating leaves after the period has passed would be expected to inactivate more infective centres than are inactivated immediately after inoculation, but this does not happen (Bawden & Kleczkowski, 1960). This, however, does not disprove the idea, because in the normal process of infection nucleic acid may, after separating from virus protein, immediately combine with some other material, which may increase its stability and resistance to UV radiation. The combination may completely protect the nucleic acid from photoreversible kind of damage by UV, as does the combination with the virus-protein in the original virus particle. Therefore, the fact that infective centres irradiated after the lag period was over could not be photoreactivated to any greater extent than when irradiated earlier (Bawden & Kleczkowski, 1960) also does not disprove the idea of the nucleic acid separating from the virus-protein *in vivo*. Moreover, the effects of UV-irradiation and of photoreactivation on the leaf capacity were so great that they might well have obscured relatively small effects on the nucleic acid if this does become free.

Siegel & Wildman (1956) concluded that, when leaves of *Nicotiana glutinosa* are irradiated within a few hours after inoculating with tobacco mosaic virus, infective centres are destroyed at the rate at which the virus is inactivated when irradiated *in vitro*, and attributed the effect of UV on lesion number solely to inactivation of the virus *in vivo*. This seems wrong, because the extent to which

infective centres are affected by UV even immediately after inoculation depends on the condition of the plant and on whether irradiated leaves are exposed to daylight or kept in darkness, although the virus itself is not photoreactivable after UV-irradiation (Bawden & Kleczkowski, 1960). Among the factors that can affect susceptibility of infective centres to UV is temperature or illumination to which the plant has been exposed for a day or so before irradiation and inoculation, the age of the plant and the season of the year (Bawden & Kleczkowski, 1960; Bawden & Sinha, 1961).

Because the inactivation lines obtained by plotting logs of percentages of lesion survival against doses of irradiation were approximately straight when irradiations were done within a few hours after inoculation with a tobacco necrosis virus or tobacco mosaic virus, Bawden & Harrison (1955) and Siegel & Wildman (1956) concluded that they had disproved the dose hypothesis of infection and established that lesions develop from cells infected by single virus particles. However, as Bawden & Kleczkowski (1960) showed, the results of the irradiation experiments neither prove nor disprove that infections are initiated by single virus particles.

The claims by Bawden & Harrison (1955) and Siegel & Wildman (1956) that changes in the shape of the inactivation lines from previously straight lines to curves of "multiple-hit" type at different times after inoculation show the times when virus particles started multiplying also seem unwarranted, because they neither take into account possible changes in the condition of infected cells, which may alter susceptibility of the cells to UV radiation, nor the fact that to prevent lesion formation larger doses of the radiation are needed some hours after inoculation than immediately after. The larger doses are obviously likely to have more effect on the capacity of cells to support virus multiplication. Moreover, the results obtained by Bawden & Harrison (1955) with a tobacco necrosis virus do not justify the conclusion that the inactivation lines do change some hours after inoculation to a curve of "multiple-hit" type. The curve they drew is not typical of a "multiple-hit" curve, and in drawing this curve the numbers of lesions were transformed according to a dilution curve that related numbers of lesions to virus concentration in the inoculum, whereas the actual numbers should have been used. This transformation enhanced the curving, which is so slight that it seems reasonable to assume that the series of inactivation lines they obtained were all almost straight and differed from each other only in their slopes. The inactivation lines published by Siegel & Wildman (1956) for tobacco mosaic virus in leaves of *Nicotiana glutinosa* do change from straight lines to curves of "multiple-hit" type, but how to interpret this is uncertain. If the change does reflect the fact that infected cells now contain more than one virus particle, then it seems that a comparable stage is not detectable in French bean leaves infected with a tobacco necrosis virus, and a "multiple-hit" curve is not typical of all virus-host combinations.

The irradiation experiments with virus-infected leaves have revealed a number of phenomena. Whether further irradiation experiments alone can explain these phenomena, however, is doubtful. At the moment irradiation does provide a method of detecting

changes in infected cells that otherwise would remain undetectable, but other methods of study will probably be needed to show the nature of these changes.

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# ISOTOPIC EXCHANGE OF PHOSPHATES IN SOIL

*Review of Experimental Techniques and Results at Rothamsted  
1952-1960*

BY

G. E. G. MATTINGLY & O. TALIBUDEEN

Paneth and Vorwerk first used radiotracers to measure the surface-exchangeable ions of solids in suspension; their now classic experiments, done in 1922, used the lead isotope, Th B, to investigate insoluble lead salts. Only much later was this technique extended to the soil-phosphate system, using growing plants to sample the soil solution (Fried & Dean, 1952; Larsen, 1952). The historical development of this subject has been reviewed earlier (Mattingly, 1957a); this article summarises laboratory experiments and pot and field experiments at Rothamsted since 1952 to evaluate soil phosphate, using the isotope  $^{32}\text{P}$ . The final section shows the extent of agreement between measurements in the laboratory and in pot experiments with soils from Rothamsted and Saxmundham Experimental Stations and Nurseries of the Forestry Commission.

## LABORATORY MEASUREMENTS

(O. Talibudeen)

### *Introduction*

In heterogeneous systems, isotopic exchange methods can be used to measure: (1) the concentration of the exchanging species at the surface of the dispersed phase, and (2) the bulk diffusion rate of the exchanging species into the dispersed phase. When applied to the soil phosphate : water equilibrium, it is difficult to separate these two measurements because adsorption sites on the soil surface and the surface chemistry of exchanging solid phosphates both vary.

These experiments were concerned with the extent and the rate of isotopic exchange in a fully dispersed soil : water system as influenced by intrinsic soil properties (e.g., carbonate content, pH, organic C content, mechanical composition, etc.) and extrinsic variables (e.g., soil : solution ratio, temperature, phosphate manuring, chelating and non-chelating organic anions, nature of the electrolyte in the equilibrating solution). The methods used are described in a series of papers (Talibudeen, 1954, 1957, 1958; Arambarri & Talibudeen, 1959, a, b, c).

Because of the complex nature of the soil-phosphate system, it is difficult to obtain unique constants for the rate of isotopic exchange which can be quantitatively related to extrinsic and intrinsic variables. However, semi-empirical constants have been obtained that are specific to each soil. To measure isotopic exchange rates,

it is convenient to maintain a constant concentration of the exchanging species in solution. In soil-phosphate equilibria this condition can be realised by maintaining equilibrium concentrations which are either very high (by adding excess soluble phosphate) or very low (by adding neutral salts). Each addition inevitably changes the soil-phosphate complex irreversibly, so that the resultant system bears little relation to the system it is desired to investigate. In our work the soil was brought to equilibrium with the phosphate concentration it would maintain in soil solution, small changes during isotopic exchange being corrected for by chemical analyses of the phosphate in solution.

Interpretation of the rate measurements presents two basic difficulties. Stable organic orthophosphates (e.g., nucleotide and phytate phosphorus) exchange isotopically at rates incomparably slower than adsorbed and particulate forms of inorganic phosphate. The isotopically exchangeable phosphate measured in the laboratory is principally related to the inorganic soil phosphate. Reliable estimates of the inorganic phosphate are therefore required to calculate the phosphate in the soil which could exchange isotopically in infinite time. Secondly, an approximate calculation shows that, on average, a *minimum* of 60 days would be required for complete isotopic exchange of the total inorganic phosphate, *if* the first-order rate constant for the slowly exchanging fraction controlled the exchange of *all* the slowly exchanging inorganic phosphate. Laboratory measurements show that small changes in specific activity of the phosphate in solution continue even after 12–14 days of continuous shaking. When these changes, measured at 48-hour intervals, are smaller than the mean error of the measurements ( $\pm 3.8\%$ ), a nearly constant figure for the isotopically exchangeable phosphate in the soil is evaluated.

The relation between the specific activity (SA) of the solution and time can be analysed semi-empirically in different ways by considering that: (1) two types of adsorption sites are responsible for isotopic exchange—those in which  $\log(\text{SA})$  and  $(\text{SA})$  are linearly related to  $(\text{time})$  and  $\log(\text{time})$  respectively; (2)  $\log(\text{SA})$  at small time-values decreases linearly with time; at large time-values  $(\text{SA})$  decreases because of self-diffusion into the solid phosphate phase and therefore as a linear function of  $(\sqrt{\text{time}})$ ; (3) the observed curvilinear decay in  $\log(\text{SA})$  with  $(\text{time})$  in laboratory experiments is composed of two or more simultaneous first-order rate processes whose rate constants are a measure of the relative ease of exchange of groups of phosphate-adsorption sites in the soil complex.

Specific activity data for over a hundred arable soils from England and Wales have been examined by the third method; the results show that at least three groups of exchanging sites are involved with half-times of exchange 10–30 minutes, 5–15 hours and 40–60 hours. The phosphate in the equilibrium solution, which is removed from the soil during equilibration, is a part of the more rapidly exchanging groups of the total isotopically exchangeable phosphate. Experimental confirmation of this is given later. In aqueous suspension (0.02M-KCl) the phosphate in solution is less than a quarter of the total isotopically exchangeable phosphate, the rapidly exchanging fraction between a quarter and a third, the

slowly exchanging group between a half and two-thirds, and with few exceptions, the middle group is less than a twentieth. The phosphate in solution, the rapidly exchanging groups and the middle group make up  $P_r$ , the phosphate adsorbed on rapidly exchanging sites;  $P_r$  expressed as a fraction of  $P_e$ , the total exchangeable phosphate, is one of the semi-empirical constants  $P_r/P_e$  used to characterise the phosphate status of a soil.

In routine work, the amount of phosphate exchanged in 20 hours is taken to be the rapidly-exchanging phosphate  $P_r$ , and that exchanged in 170 hours as the "total" isotopically exchangeable phosphate  $P_e$ . Routine  $P_r$  values are over-estimated on average by about 25% of  $P_{\text{slow}}$  (where  $P_{\text{slow}}$  is the slowly-exchanging phosphate with an average half-time of exchange of 50 hours).  $P_e$  is usually only slightly under-estimated, because about 98% of the equilibrium  $P_{\text{slow}}$  exchange is completed in 170 hours. Hence  $P_r/P_e$  is an approximate value in routine determinations and less accurate than that derived from a full-scale rate-analysis.

The second "constant" used is the ratio  $P_e/P_t$ , where  $P_t$  is the total soil phosphate determined by perchloric acid digestion. This is a less sensitive and less accurate index than  $P_r/P_e$ , principally because  $P_t$  includes the inert and isotopically non-exchangeable organic phosphate, which cannot be estimated accurately.

#### *Materials*

Soils from four sources were used: (a) the classical plots at Rothamsted; (b) the nurseries of the Forestry Commission (Benzian, 1959); (c) N.A.A.S. experimental plots at Shardlow, Nr. Derby (Blood, 1957); (d) calcareous soils from selected arable sites supplied by the Soil Survey of England and Wales.

#### *Extrinsic factors affecting the isotopically exchangeable phosphate*

Various procedures have been suggested in the last 10 years to measure the isotopically exchangeable phosphate in soils. The aim has been to develop a routine laboratory procedure which gives values that not only correlate well with those in pot experiments but are equal to them. Before establishing a routine procedure it was necessary to evaluate quantitatively the influence of external variables on the isotopically exchangeable soil phosphate and to avoid any which significantly altered this numerical equality. The degree of success attained is discussed below (p. 261).

(a) *Soil : solution ratio.* The phosphate concentration in solution increased with soil : solution ratio, but the isotopically exchangeable phosphate measured at any one time *decreased* at a fixed and optimum rate of agitation (Talibudeen, 1954). The effect of increasing soil : solution ratios diminished at larger reaction times; the measurements indicated that soil : water ratios below 2 g. : 100 ml. did not alter the isotopically exchangeable phosphate.

(b) *Concentration of soluble phosphate added with  $^{32}\text{P}$  for measuring isotopically exchangeable phosphate.* Most methods suggested for determining isotopically exchangeable phosphate use  $^{32}\text{P}$  solutions of low specific activity (high phosphate content), and although good correlation is obtained with plant-derived indices, numerical equality is rarely observed. Experiments on a neutral Rothamsted soil



(Highfield) showed that, in the range of  $3.2 \times 10^{-7}M$  to  $1.5 \times 10^{-4}M$  phosphate, higher concentrations significantly decreased the isotopically exchangeable phosphate at any one reaction time (Talibudeen, 1957). To obtain a specific soil index it is necessary to add  $^{32}P$  in a phosphate solution which does not appreciably alter the phosphate level in the equilibrium soil solution.

(c) *Organic anions.* Moderately high concentrations of anions (e.g., citrate and bicarbonate) which chelate cations in combination with soil phosphate are commonly used for evaluating phosphate residues; non-chelating anions (e.g., acetate) have also been used for this purpose. Isotopic exchange measurements are difficult with soils of very low phosphate status, because the phosphate concentrations to be measured in solution are so small. Very low concentrations of citrate ions were used to raise this phosphate concentration (Talibudeen, 1958). Two Rothamsted soils showing moderate response to phosphate were examined to estimate the influence of chelating and non-chelating organic anions at a  $0.001M$  level (Arambarri & Talibudeen, 1959a). Table 1 shows that soil pH directly influences the interaction of organic anions. The chelating citrate ion increases the total  $P_e$  in the slightly acid soil, but diminishes total  $P_e$  in the calcareous soil, although the rate of

TABLE 1

*Influence of soil pH and chelating constants on the interaction of 0.001 Molar concentrations of organic anions with the isotopically exchangeable phosphate in Rothamsted soils.*

Organic anion	Chelating constant for Ca log ( $K_{Ca}$ )	Isotopic exchangeability of soil phosphate					
		Highfield pH 6.7			Exhaustion land (superphosphate plot) pH 7.5		
		$P_e$	$P_r/P_e$	$R_{slow}^*$	$P_e$	$P_r/P_e$	$R_{slow}^*$
No organic anion ...	—	11.3	0.284	0.013	11.5	0.331	0.020
Phenyl-barbiturate	0.66	9.1	0.486	0.040	7.1	0.491	0.020
Citrate ...	3.15	16.3	0.760	0.044	9.5	0.476	0.037

\*  $R_{slow} \equiv$  1st-order rate constant of slowly exchanging phosphate, in mg.  $P/100$  g. soil/hour.

exchange  $R_{slow}$  of phosphate is increased equally in both soils. In contrast, the non-chelating barbiturate ion decreases total  $P_e$  in both soils, increasing  $R_{slow}$  in the acid soil but not altering  $R_{slow}$  in the calcareous soil. These figures illustrate some of the difficulties in using foreign ions, even in such low concentrations, to aid isotopic exchange determinations of the soil phosphate;  $P_e$  values with and without  $0.001M$ -citrate are compared below (p. 261).

(d) *Nature of electrolyte in equilibrating solution.* Although isotopic exchange measurements in soil:water suspensions avoid specific ion interactions, they are impracticable in most soils. To ensure rapid and efficient separation of soil and solution and to provide a constant ionic strength without unduly diminishing the equilibrium phosphate concentration in solution,  $0.02M$ -KCl solution was used. Experiments with NaCl, KCl and  $CaCl_2$  solutions show that in strong electrolytes the valency of the cation has little effect on the total  $P_e$  (Arambarri & Talibudeen, 1959b).

(e) *Temperature.* Equilibrium phosphate concentrations in soil solution have a positive temperature coefficient, for which Aslyng (1950) gave an average value of 1–2% per °C. The temperature coefficient of the total labile phosphate for calcareous soils between 25° and 45° was between 0.8 and 6.6%, which emphasises the need to control temperature within  $\pm 1^\circ$  in measurements of isotopically exchangeable phosphate (Arambarri & Talibudeen, 1959c).

*The influence of intrinsic soil properties on the extent and rate of isotopic exchange of soil phosphate*

The soils examined were divided into two broad groups—(a) calcareous soils containing >0.1% carbonate with pH values >7.2 in 0.01M-CaCl<sub>2</sub> or 0.02M-KCl; (b) non-calcareous soils containing <0.1% CaCO<sub>3</sub> in the pH range 3.7–7.0. Phosphate-retaining mechanisms and soil properties which would influence the extent and distribution of the soil phosphate in the two groups are quite different. The only common factor affecting soil phosphate distribution is the mechanical composition of the soil.

(a) *Calcareous soils.* The phosphate-retaining phase of primary importance is the carbonate fraction; this is conventionally referred to in soil analysis as *calcium* carbonate, although it may also often contain the carbonates of Mg, Fe, Mn and Zn.

Isotopic exchange measurements at Rothamsted on more than 50 soils from the United Kingdom and Spain showed that, although the rapidly exchanging phosphate,  $P_r$ , was unrelated to the carbonate content, the ratios  $P_r/P_e$  and  $P_e/P_t$  both increased linearly with decreasing carbonate content (Arambarri, 1960). Later work on four groups of soils selected from arable sites, where the carbonate and other mineral constituents were of similar origin within each group, showed that the ratio  $P_r/P_e$  decreased with increasing

TABLE 2

*Decrease in the distribution of rapidly exchanging phosphate with increasing carbonate content in different soil groups (four soils in each group)*

Origin of carbonate	Decrease in $P_r/P_e$ per g. CaCO <sub>3</sub>
Upper/Middle Chalk	$1.6 \times 10^{-3}$
Oolitic Limestone A	4.2
Magnesian Limestone	5.5
Lower Lias	9.3

carbonate content. In two of these soil groups with a large range of carbonate contents this relationship consisted of two linear portions. Up to 10% carbonate content the decrease in  $P_r/P_e$  was larger; at higher carbonate contents the change in  $P_r/P_e$  per unit change in carbonate content was less. Within the accuracy of measurement, the inverse linear relation between  $P_r/P_e$  and carbonate content was different for each soil group (Table 2). The surface exchangeable calcium (measured by isotopic exchange using the <sup>45</sup>Ca isotope in a "difference" method), in soils with similar carbonate contents from six sites, showed that  $P_r/P_e$  was

inversely proportional to the surface-calcium in the carbonate fraction. Surface areas, based on the surface density of  $\text{Ca}^{2+}$  ions on calcite surfaces, suggested that the "carbonate" crystallites in calcareous soils have an "effective" diameter of  $0.25 \mu$ , and therefore carbonate particles in size fractions larger than this diameter are very porous. In soils containing carbonate with the lowest specific surface, the first-order rate constant of the slow-exchanging phosphate fraction is at least three times as great as in soils with carbonate of high specific surface (Table 3). This table also illus-

TABLE 3

*Relation between the first order rate constant of the slowly exchanging surface phosphate groups and the specific surface of the carbonate fraction in soils with similar carbonate contents (25–35%  $\text{CaCO}_3$ ) of different geological origin*

Carbonate	Specific surface (sq. m./g. $\text{CaCO}_3$ )	$R_{\text{slow}}$ (mg. P/100 g. soil/hour)
Coral Rag and Coralline Oolite ...	2.1	0.065
Portland Limestone ...	2.8	0.088
Magnesian Limestone ...	3.9	0.036
Chalk Marl Limestone ...	4.7	0.023
Lake Marl Limestone *	7.9	0.008
Synthetic Calcite ( $25 \mu$ diameter) †	0.15	—

\* Containing 52%  $\text{CaCO}_3$ .

† *Rep. Rothamst. exp. Sta. for 1958, p. 50.*

trates the variation in specific surface of the soil carbonate of different geological origins together with that of synthetic calcite. Isotopic exchange measurements show that added phosphate reverts to less-exchangeable forms at rates and in amounts that are directly proportional to: (1) the carbonate content (and thus to the total surface exchangeable Ca) in soils from one group containing carbonate of the same origin; (2) the surface-exchangeable calcium per g.  $\text{CaCO}_3$  (or the specific surface) of the carbonate fraction in soils of similar carbonate content but of different geological origin.

Also, in each soil,  $P_e$  (as the quantity of phosphate exchanging) is linearly related to the phosphate in solution (as the intensity of phosphate), for the untreated soil and the phosphate-treated soil after 1, 2 and 6 months.

(b) *Non-calcareous soils.* Increase in soil acidity increases the aluminium (and iron) concentration (in particular, the activity of the  $\text{Al}^{3+}$  ion) in the soil solution, and the surface concentration of positively charged sites on soil minerals. The increase in  $\text{Al}^{3+}$  activity lowers the phosphate concentration in solution in several pairs of soils, where the soil, treated with equal amounts of superphosphate, has been maintained at different pH levels (Chakravarti, 1959). Table 4 shows that, in addition, decreasing soil pH affects  $P_e$  and  $P_r/P_e$  in the same way. The effect of soil pH on the isotopically exchangeable phosphate clearly illustrates the need for a reliable method for measuring the concentration of these positively charged sites on the soil surface. This may help to define the composition of surface-adsorbed layers formed in acid soils treated

with average agricultural levels of superphosphate. Table 4 also illustrates how the organic carbon content of the soil modifies the effect of soil pH on the water-soluble phosphate and the isotopically exchangeable phosphate. Thus, in a soil with less organic carbon the water-soluble phosphates and  $P_e$  decrease more with decreasing pH than in a soil with more organic carbon. This qualitatively indicates the blocking of positive adsorption sites by organic molecules or anions in soils with high organic carbon content, especially at lower pHs.

TABLE 4  
*Effect of soil pH and organic carbon content on water soluble phosphate and isotopically exchangeable phosphate*

Soil	% Organic carbon *	pH	Total soluble aluminium phosphate ( $\times 10^{-6}$ Molar)	Soluble phosphate	$P_e$ (mg. P/ 100 g. soil)	$P_r/P_e$
<i>Park Grass</i>						
Plot 4(i) Unlimed	3.07	5.2	5.1	17.0	36.5	0.647
4(i) Limed ...	3.05	6.8	1.2	25.0	41.4	0.727
<i>Shardlow Unmanured plots</i>						
Plot A5 ...	1.58	4.0	28.0	0.58	17.6	0.472
C5 ...	1.67	5.0	3.1	1.5	21.3	0.545
E5 ...	1.87	6.0	2.6	4.1	28.8	0.392(?)
<i>Broadbalk Section V, Plot 8</i>						
Sample 2 ...	1.19	5.3	—	4.4	22.9	0.477
3 ...	1.21	6.0	—	5.7	21.6	0.601
5 ...	1.19	7.5	—	9.7	30.7	0.635

\* Walkley-Black Method (with acknowledgements to J. M. d'Arifat).

Cropping modifies the contrasts in  $P_r$  and  $P_e$  between unmanured and phosphate-treated soils, especially in soils with higher pHs from which much more of the added phosphate is removed. However, the effect of soil pH on the nature of phosphate residues can be evaluated by isotopic exchange measurements. Table 5 gives ratios calculated from differences in the  $P_r$ ,  $P_e$  and  $P_t$  values for untreated and phosphate-treated soils from Shardlow at pH 4.0, 5.0 and 6.0. Thus,  $\Delta P_r/\Delta P_e$  and  $\Delta P_e/\Delta P_t$  values indicate that at lower pHs the residual phosphate is held in less exchangeable forms.

TABLE 5  
*The effect of pH on the isotopic exchangeability of phosphate residues in soils from N.A.A.S. Centre, Shardlow*

Soil pH	$\Delta P_r/\Delta P_e$	$\Delta P_e/\Delta P_t$
4.0	0.436	0.700
5.0	0.805	0.828
6.0	0.831	0.722(?)

(c) *Phosphate manuring*. Much new information has been added to that already published (Talibudeen, 1958). It shows that:

(a) When water-soluble forms of phosphate are added to acid or calcareous soils the absolute values of  $P_r$  and  $P_e$ , as well as the proportion of added phosphate on rapidly exchanging sites (defined

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by the ratios  $P_r/P_e$  and  $P_e/P_t$ , are significantly increased. In any one soil, this increase depends on: (1) the level of phosphate added; (2) the phosphate status of the soil initially; (3) the length of time the added phosphate has been in contact with the soil; and (4) in cropped soils the amount of phosphate removed from the soil by the crop before sampling. The effects of manuring and cropping are described below (pp. 257-259). The influence of the first three factors is briefly illustrated.

The  $P_r/P_e$  index is sensitive enough to detect the *residual* effect of 0.5 and 1.0 cwt.  $P_2O_5$ /acre (added as superphosphate) after 2 years, even when superimposed on the residual effect of previous manuring (Table 6). Larger changes in  $P_r/P_e$  have also been

TABLE 6

*Isotopic exchangeability of soil phosphate in Exhaustion Land two years \* after a dressing of 0.5 and 1.0 cwt.  $P_2O_5$  per acre as superphosphate*

Previous manuring †	Plot 5 No phosphate Ammonium salts (1856-1901)			Plot 9 Superphosphate (1856-1901)		
	0	0.5	1.0	0	0.5	1.0
Cwt. $P_2O_5$ /acre as superphosphate in 1957						
Crop				$P_r/P_e$ values		
Kale ... ..	0.540	0.570	0.595	0.589	0.605	0.620
Swede ... ..	0.490	0.557	0.576	No sampling		

\* One year's cropping (kale or swede) followed by ploughing and one year's fallow.

† For full details, see Warren & Johnston, 1960.

observed (together with increases in  $P_r$  and  $P_e$ ) under two very different sets of conditions (Talibudeen, 1958). In laboratory incubations when the soil was dressed with 10 mg. P/100 g., the isotopic exchangeability of the residue after 3 months' incubation was lower on the unmanured soil which also had the smaller  $P_r/P_e$  value (Table 7).

TABLE 7

*Isotopic exchangeability after three months incubation at field capacity of phosphate added (10 mg. P/100 g. soil) to Exhaustion Land soils in relation to their phosphate status*

Manurial history	Phosphate status of untreated soil $P_r/P_e$	Isotopic exchangeability of 10 mg. P addition after three months	
		$\Delta P_r/\Delta P_e$	$\Delta P_e/\Delta P$
No manure ... ..	0.532	0.677	0.65
FYM (1856-1901) ... ..	0.689	0.743	0.70
Superphosphate (1856-1901)	0.666	0.771	0.70

(b) When very insoluble forms of phosphate (e.g., apatites) are added to calcareous soils little change in isotopic exchangeability of the soil phosphate can be detected (Talibudeen, 1958). However, the isotopic exchangeability of phosphate residues in acid soils

treated with rock phosphate or basic slag are not very different from superphosphate residues (Table 8).

(d) *Nature of exchangeable cations in the soil.* The isotopic exchangeability of soil phosphate is principally affected by the factors described above, although the exchangeable cations play a secondary but significant part in influencing it. In non-calcareous soils divalent exchangeable cations decrease the rate of exchange of the slowly exchanging phosphate without altering its extent;

TABLE 8

*Isotopic exchangeability of phosphate residues from different sources in soils (pH 4.9–5.4) from Wareham Nursery, sampled in 1955*

Phosphate source	$\Delta P_r$	$\Delta P_e$	$\Delta P_i$	$\Delta P_r/\Delta P_e$	$\Delta P_e/\Delta P_i$
Gafsa rock phosphate	2.25	2.70	7.3	0.833	0.370
Basic slag ...	1.60	1.78	5.0	0.900	0.356
Superphosphate ...	1.70	1.85	4.9	0.920	0.378

this rate of exchange is also less sensitive to temperature than with soil saturated with monovalent cations. Thus, manurial treatments which shift the cation balance in the soil in favour of monovalent cations increase the rate of exchange of the slowly exchanging phosphate. It is suggested that this is caused by the decrease in  $Al^{3+}$  activity at the soil : water interface when changing from a divalent-cation-dominated soil to a monovalent one (Arambarri & Talibudeen, 1959b).

#### FIELD AND GREENHOUSE EXPERIMENTS (G. E. G. Mattingly)

##### *Radiation effects from $^{32}P$*

Conflicting reports have appeared on the magnitude and reproducibility of the effects of radiation on the growth of plants. Most workers have detected slight alterations in growth with both low and high levels of  $^{32}P$  (see Mattingly, 1957a for references). In preliminary experiments in the greenhouse using ryegrass, dry weight and phosphorus uptake were the same when either 5 and 10  $\mu C$  or 10 and 50  $\mu C$   $^{32}P$ /pot were compared. However, less fertiliser phosphorus was taken up at the higher levels of radioactivity. There was no difference between effects of  $^{32}P$  at different  $^{31}P$  levels, or with different soils (Mattingly, 1957b). With barley,  $^{32}P$ -labelled superphosphate gave the same yield of grain, straw and roots in the greenhouse and the same distribution of phosphorus in the crop as an equivalent amount of commercial superphosphate (Mattingly & Widdowson, 1958b). The effects of  $^{32}P$  on growth and fertiliser uptake in greenhouse experiments were always small but reproducible, probably because the fertiliser was mixed uniformly throughout the soil. In field experiments, using superphosphate labelled with 0.7–1.4 mC  $^{32}P$ /g. P, yields of barley and uptakes of phosphorus in the field were slightly lower than with commercial superphosphate (Mattingly & Widdowson, 1958b).

### *Field experiments*

The main purpose of the field experiments using  $^{32}\text{P}$ -labelled superphosphate was: (a) to establish whether the increased yields that usually result from applying water-soluble phosphates near the seed of cereals could be attributed to greater uptake of soil phosphorus, of fertiliser phosphorus, or both; (b) to measure by isotope dilution the total "pool" of isotopically exchangeable phosphate in the soil at different stages of growth and the increase in this "pool" from applying different non-radioactive phosphate fertilisers.

*The uptake of soil phosphorus by barley.* When barley grew rapidly, more total phosphorus and more phosphorus from the soil was removed in 26 days from plots receiving superphosphate than from plots without phosphate fertilisers. This effect was largest when the superphosphate was drilled with the seed; the *extra* phosphorus in the plants taken up from soil was then comparable with the phosphorus taken up from the fertiliser. Barley given broadcast superphosphate removed smaller amounts of *extra* soil phosphate. This effect did not persist until the crop was harvested, when, in seven out of eight comparisons, *less* soil phosphorus had been taken up when superphosphate was applied. Very similar results have been obtained elsewhere with wheat (Mitchell, 1957) and with oats (Verma *et al.*, 1959).

There are three possible explanations of the changes in uptake of soil phosphorus during growth: (i) radioactive superphosphate damages plants; (ii) superphosphate alters the growth pattern of roots; (iii) superphosphate "blocks" the removal of soil phosphorus. The first is unlikely, because increasing levels of  $^{32}\text{P}$  usually decrease fertiliser uptake without greatly altering the total phosphorus removed by the crop. Consequently, the uptake of soil phosphorus should *increase* on applying a radioactive superphosphate. There is some evidence (Cooke, 1954) that the root system of peas, and presumably of other crops, is considerably more diffuse without phosphorus fertilisers than when superphosphate is either drilled or broadcast. This suggests that the roots of unmanured plants are in contact with a larger soil surface than those of plants receiving superphosphate. The exchangeable phosphate sites on soil surfaces may also be "blocked" by fertiliser phosphates, as in laboratory experiments (Talibudeen, 1957), which would further limit the removal of soil phosphate.

*Isotope dilution with non-radioactive phosphate fertilisers.* Experiments with  $^{32}\text{P}$ -labelled fertilisers not only give information on the uptake of phosphorus from the fertiliser and soil but they also estimate the total "pool" of soil phosphorus sampled by the crop at different stages of growth. In the greenhouse, when soil and fertiliser are well mixed, the ratio of  $^{32}\text{P}/^{31}\text{P}$  in the crop decreases only slightly during growth, as progressively more soil phosphate exchanges. In the field, however, this ratio falls more rapidly as roots take up phosphate from soil which is not mixed with the labelled fertiliser.

The total "pool" of soil phosphorus sampled by barley and fodder beet in the presence of drilled superphosphate (i) increased during growth, (ii) varied between the crops, and (iii) varied very

greatly in different years on the same site (Mattingly & Widdowson, 1958a).

The total "pool" sampled at harvest on one site was 37 and 55 lb. P/acre for fodder beet and barley respectively; both values are larger than the total amount of phosphate removed by the crops. The smaller "pool" with fodder beet indicates that the root system of this crop does not remove phosphate from soil far from the fertiliser zone. When commercial non-radioactive phosphate fertilisers were broadcast in these experiments the increase in total labile phosphate in the soil was equal for fodder beet to the amount of phosphorus applied as superphosphate or dicalcium phosphate. With barley the increase was less than the total applied. Gafsa rock phosphate did not significantly increase either the "pool" of soil phosphate sampled or the yield of the crop in any experiment.

The conclusions from these experiments confirm and provide some quantitative explanations for results in other field experiments on fertiliser placement. Barley produces higher yields when superphosphate is drilled with the seed than when broadcast (Crowther, 1945). The experiments with  $^{32}\text{P}$  show that (i) a higher proportion of phosphate is taken up from drilled than broadcast superphosphate, (ii) drilled superphosphate usually increases uptake of soil phosphate in the early stages of growth, and (iii) only part of the phosphate in superphosphate, when applied broadcast, increases the "pool" of labile soil phosphate sampled by the crop. In contrast, placing superphosphate for sugar beet, which has a similar root system to fodder beet, is very little more effective than broadcasting (Prummel, 1957), probably because the "pool" of phosphate sampled is restricted to the soil which is mixed with the broadcast fertiliser.

There is a fundamental difference between the interpretation of isotope dilution experiments in the field and in the greenhouse. In the greenhouse the root system is restricted to a known weight of soil. The amount of phosphate that exchanges *in unit weight of soil* can be calculated from the  $^{32}\text{P}/^{31}\text{P}$  ratio in the crop.

In field experiments the amounts of soil phosphorus accessible *per acre* to the crop can be calculated from the  $^{32}\text{P}/^{31}\text{P}$  ratio. However, phosphorus derived by the crop from soil mixed with the fertiliser cannot be directly distinguished from that derived from lower layers of soil, unless the limiting value of the  $^{32}\text{P}/^{31}\text{P}$  ratio for unit weight of soil is known from a greenhouse experiment. In a field experiment in 1953, when barley grew very rapidly, all the dilution of fertiliser phosphorus by  $^{31}\text{P}$  from the soil could be accounted for at harvest by the isotopically exchangeable phosphate estimated, from greenhouse experiments, to be present in the plough layer. In 1954, when the crop grew more slowly, the  $^{32}\text{P}/^{31}\text{P}$  ratio was very small at harvest, and indicated that about one-half of the  $^{31}\text{P}$  in the crop had come from below the layer of soil with which the fertiliser phosphate was mixed by harrowing (Mattingly & Widdowson, 1958b).

*Evaluation of residues of phosphate fertilisers by isotope dilution.* Similar methods using  $^{32}\text{P}$  have been used to estimate the residual value of superphosphate in a field experiment with barley. The value in 1955 of the residues of superphosphate applied in 1954 was estimated from (a) yield, (b) phosphorus uptake and (c) changes in



the  $^{32}\text{P}/^{31}\text{P}$  ratio in the crop. Six weeks after sowing, all three methods of evaluation showed that the residues were equivalent to about one-quarter as much superphosphate broadcast in 1955, and yields of grain at harvest also gave this value. Evaluations using phosphorus uptake and changes in the  $^{32}\text{P}/^{31}\text{P}$  ratio in the crop were higher and equal to 40–50% of the amount of superphosphate applied in 1955. These results suggest that at harvest the root system of barley reached residues of superphosphate that had been buried by ploughing. The phosphorus taken up from these residues ("luxury uptake") did not increase yield but decreased the ratio  $^{32}\text{P}/^{31}\text{P}$  in the crop (Mattingly & Widdowson, 1956).

#### *Greenhouse experiments*

Preliminary work (Mattingly, 1957b) established a standard technique for greenhouse experiments. A solution of monocalcium phosphate containing 2.0 mg. P, labelled with  $10\ \mu\text{C}\ ^{32}\text{P}$ , was mixed mechanically with 400 g. air-dry soil and 200 g. coarse quartz per pot. Perennial ryegrass (*Lolium perenne*, S23 strain), used as the test crop in most experiments, was cut at intervals of 3–4 weeks. This level of radioactivity was enough to measure the  $^{32}\text{P}/^{31}\text{P}$  ratio in the grass up to 16 weeks from sowing. A few experiments were done using 100 g. soil labelled with 0.5 mg. P containing  $5\ \mu\text{C}\ ^{32}\text{P}/\text{pot}$ .

For the first cut of grass, the total amount of phosphate that exchanges per 100 g. soil during the greenhouse experiment is given by the equation

$$A = 0.5 \left\{ \frac{(^{32}\text{P}/^{31}\text{P}) \text{ fertiliser}}{(^{32}\text{P}/^{31}\text{P}) \text{ crop}} - 1 \right\}$$

For several cuts of grass  $(^{32}\text{P}/^{31}\text{P})_{\text{crop}}$  is replaced by  $(\sum_1^n ^{32}\text{P} / \sum_1^n ^{31}\text{P})$ , where  $\sum_1^n$  is the sum of  $^{31}\text{P}$  or  $^{32}\text{P}$  removed in  $n$  cuts.

*Comparison of phosphate fertilisers by isotope dilution.* Experiments, similar in principle to those in the field, were done in the greenhouse to evaluate different phosphate fertilisers. Commercial, non-radioactive phosphate fertilisers were added to soils and compared with superphosphate by measuring (i) yield, (ii) phosphorus uptake, and (iii) changes in the ratio  $^{32}\text{P}/^{31}\text{P}$  in the crop.

All three methods of evaluation showed that powdered dicalcium phosphate and silico-phosphate were equivalent to superphosphate in the greenhouse. However, rock phosphates gave much higher values by the isotope dilution method than from measurements of yield and phosphorus uptake, probably because  $^{32}\text{P}$  was distributed unevenly in soil around particles of rock phosphate which dissolve slowly (Mattingly & Widdowson, 1956).

*Measurement of the residual value of phosphate fertilisers.* The principle of isotope dilution with  $^{32}\text{P}$ -labelled orthophosphate has been used by several workers to estimate the residual phosphate in soil that remains isotopically exchangeable in greenhouse experiments. Table 9 gives estimated changes since 1901 in the "A" value of soils from the Hoosfield Exhaustion Land experiment at Rothamsted. Details of this experiment are given elsewhere (Warren, 1956; Warren & Johnston, 1960).

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The values in line *a* were calculated, assuming 1 acre of soil weighs 2 million lb., from the average removals of phosphate by barley in the field reported by R. G. Warren in *Guide to the Experimental Farms* (Rothamsted, 1959, p. 26). "A" values in 1901 are not known, but were estimated from measurements on other plots at Rothamsted. Values in line *c* were determined in a greenhouse experiment.

TABLE 9  
Removal of P by cropping and changes in "A" values on  
Hoosfield Exhaustion Land, 1901-1957  
(All results in mg. P per 100 g. soil)

	Plot 1 (no P; no K)	Plot 3 (FYM before 1901)	Plot 9 (Superphosphate before 1901)
(a) P removed by cropping 1901-1957 ...	12	26	23
(b) "A" value in 1901 (estimated) ...	10	21	21
(c) "A" value in 1957	3	10	9
(d) Decrease in "A" between 1901 and 1957 ...	7	11	12

Although the results involve some assumptions, they indicate that during 60 years only one-half of the phosphate removed by cropping came from the isotopically exchangeable "pool". The remainder has been derived from soil phosphate in an unknown chemical form that was not isotopically exchangeable in 1901.

In short-term field experiments the amount of phosphate taken up by the crops almost equals the decrease in "A". The "A" values of soils from the Four-Course rotation at Rothamsted decreased each year after applying superphosphate, but remained constant (at lower values than the superphosphate plots) after applying Gafsa rock phosphate. Rock phosphate appears to dissolve slowly in this calcareous soil, at a rate approximately equal to the rate at which phosphate is removed by the crops in the rotation (Mattingly, 1957b).

The increase in total phosphate content of soils ( $\Delta P_t$ ) from applying phosphate fertilisers can be determined by chemical analysis. The quantity of the residual phosphate in calcareous soils that is isotopically exchangeable has been measured in some greenhouse experiments from the increase in "A" values ( $\Delta A$ ) on plots receiving phosphate fertilisers. The values obtained for the ratio  $\Delta A/\Delta P_t$  vary from 20 to 70% of the total residual phosphate (*Rep. Rothamst. exp. Sta.* for 1957, p. 61; for 1959, p. 50). This is greater than the range 26-56% quoted by Olsen *et al.* (1954) for calcareous soils in the U.S.A.

Some of the most important factors influencing the amount of residual phosphate that remains isotopically exchangeable are (i) pH, (ii) the quantity of phosphate removed by cropping, (iii) soil type, and (iv) the form of nitrogen fertilisers applied for long periods with superphosphate. The relative importance of (i), (ii) and (iv) is difficult to determine because they are interrelated, but the most

important single factor, on any soil type, appears to be removal of phosphate by cropping. This is illustrated below using soils from a rotation experiment on calcareous Boulder Clay at Saxmundham, details of which are given by Cooke *et al.* (1958).

TABLE 10

*Effect of cropping in the field on the percentage of the residual phosphate ( $\Delta A/\Delta P_t$ ) that is isotopically exchangeable in calcareous soils from Saxmundham*

Field treatment	Mean yields of barley 1899-1956 (bushels/acre)	$\Delta A/\Delta P_t$ (%)
P ... ..	21	73
PK ... ..	23	71
NP ... ..	34	64
NPK ... ..	36	56

(P = 2 cwt. superphosphate/acre; N = 2 cwt. sodium nitrate/acre; K = 1 cwt. of potassium chloride/acre.)

Yields and amounts of phosphate removed by the crops were low without added nitrogen, and more of the residual phosphate in these soils now remains isotopically exchangeable.

Saxmundham soils have higher  $\Delta A/\Delta P_t$  values than the calcareous soils at Rothamsted, on which this ratio varies from about 20 to 50%. The highest values at Rothamsted are on plots that have, at some time in their history, been acid from applying ammonium sulphate. The average value for  $\Delta A/\Delta P_t$  in acid and very acid soils taken from nine field experiments in Great Britain and Japan was 70% (*Rep. Rothamst. exp. Sta.* for 1959, p. 49).

There are several limitations to experiments of this type using soil samples taken from field experiments. When the amounts of phosphate applied as fertiliser are small compared with total soil phosphate,  $\Delta P_t$  is determined from the difference between two large quantities. In the experiments so far completed increases in phosphorus content shown by analysis have generally agreed closely with increases calculated by assuming the plough layer of 1 acre of soil weighs between 2.0 and 2.5 million lb.

The experiments referred to above show how much of the phosphate applied to soils as superphosphate remains isotopically exchangeable. They do not, however, give any direct evidence of the chemical form in which the phosphate residues exist in soil. Phosphate ions are probably adsorbed, rather than precipitated, because so large a proportion remains isotopically exchangeable.

*Relationships between "A" values and crop growth.* Yield of ryegrass, grown in the greenhouse and given optimum N, K and minor elements, is exponentially related, and uptake of phosphate by the crop linearly related, to the "A" values of soils. The equations below give typical relationships between yield ( $y$ ) of ryegrass and the "A" values ( $x$ ) for two groups of soils with very different physical properties:

Rothamsted soils (calcareous; 20-30% clay)  

$$y = 17.3 - 25.2e^{-0.161x}$$

Forest Nursery soils (acid; 1-10% clay)  

$$y = 17.5 - 18.4e^{-0.161x}$$

The maximum yields (17.3–17.5 g./pot) were the same for both groups when the experiments were continued for 4 months; this suggests that growth in the greenhouse was limited by phosphate and not by physical differences between the soils. The yield, per unit of isotopically exchangeable phosphate ("A"), was much greater on the lighter acid soils from nurseries of the Forestry Commission than on the heavier soils from Rothamsted. The more rapid growth of grass on the nursery soils has been attributed to the higher phosphate concentration, measured in 0.01M-CaCl<sub>2</sub>, they maintain per unit of exchangeable phosphate (*Rep. Rothamst. exp. Sta. for 1957*, p. 59).

Table 11 shows the relationships between the uptake of phosphorus by ryegrass in three to four months and "A" values, found in four greenhouse experiments.

TABLE 11

*Linear regression equations and correlation coefficients between uptake of phosphorus by ryegrass in the greenhouse (y) and "A" values of soil (x)*

(All results in mg. P/100 g. soil)

Experiment	Brief description of soils	No. of soils	Regression equation	Correlation coefficient
P9	Rothamsted; calcareous	9	$y = 0.546x - 0.52$	0.998
	Forest Nurseries; acid	12	$y = 0.668x - 0.37$	0.986
P12	Rothamsted; calcareous	7	$y = 0.217x - 0.42$	0.977
	Saxmundham; calcareous	9	$y = 0.290x - 0.41$	0.991
P11	Acid and calcareous arable soils from C. and E. England	15	$y = 0.418x - 1.31$	0.870
P14	Acid and very acid soils from England, Wales, N. Ireland and Japan	24	$y = 0.335x - 1.15$	0.879

Phosphorus uptake and "A" are very highly correlated, especially so with soils from the same location. The differences between the regression lines for the different groups of soils in experiments P9 and P12 were both significant. The greater release of phosphate from the forest nursery soils agrees with the more rapid growth on them. No satisfactory explanation has yet been found for the differences between calcareous soils of similar clay content and composition derived from the Clay-with-Flints at Rothamsted and the Boulder Clay at Saxmundham in which the ratio  $P_r/P_e$  (Table 13) and phosphate concentrations in 0.01M-CaCl<sub>2</sub> are similar.

The high correlations in these experiments, and the fact that there is no outstanding discrepancy, may be partly explained by the technique used. In all experiments a "starter dose" of phosphate (0.5 mg. P/100 g. soil) was mixed with the soil. Although this is small compared with the "A" values of most soils (5–25 mg. P/100 g.), it may have maintained the concentration of phosphate high enough after germination for the root system to develop. This explanation is supported by evidence with both acid and calcareous soils that the *initial* rate of growth of grass in the greenhouse on soils with the same "A" value is most rapid when the phosphate concentrations in 0.01M-calcium chloride are high (*Rep. Rothamst. exp. Sta. for 1957*, p. 59; for 1959, p. 44).

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The initial rate of growth of grass depends principally on phosphate potential, as predicted by Schofield (1955), but total yield and uptake of phosphorus increase with "A" and can, on some soils, be almost independent of phosphate potential. However, in Rothamsted soils phosphate potential (phosphate concentration) and quantity ("A") are closely correlated, so that either measurement predicts their phosphate status satisfactorily.

## RELATIONSHIPS BETWEEN LABORATORY AND GREENHOUSE MEASUREMENTS OF ISOTOPICALLY EXCHANGEABLE PHOSPHATE

*Correlation of "A" and  $P_e$* 

Table 12 summarises the correlations obtained between greenhouse ("A") and laboratory ( $P_e$ ) values for isotopically exchangeable phosphate in arable soils.

TABLE 12

*Summary of relationships between "A" and  $P_e$  measured in 0.02M-KCl or in 0.02M-KCl + 0.001 NH<sub>4</sub> citrate for arable soils*

No. of soils	Description	Electrolyte	Regression equation	Correlation coefficient
37	Acid, neutral and calcareous	0.02M-KCl	$A = 1.067P_e + 0.21$	+0.985
12	Acid	0.02M-KCl + 0.001M-NH <sub>4</sub> citrate	$A = 0.771P_e + 0.09$	+0.966
12	Calcareous	0.02M-KCl + 0.001M-NH <sub>4</sub> citrate	$A = 1.238P_e + 1.18$	+0.993

The 37 soils tested had clay contents between 1 and 30%, pH values (in 0.01M-CaCl<sub>2</sub>) between 3.9 and 7.6, and  $P_e$  values between 1 and 32 mg. P/100 g. Values of "A" and  $P_e$ , measured in 0.02M-KCl, were very highly correlated, and "A" was numerically about 7% higher than the  $P_e$  values determined by the routine method. The regression equation shows that the total "pool" of soil phosphate sampled by ryegrass grown for 3-4 months in the greenhouse can be estimated quantitatively by isotopic exchange in the laboratory in about 7 days. The close correlations previously established between "A" and phosphorus uptake from soils (Table 11) will, therefore, also hold for  $P_e$  and phosphorus uptake in the greenhouse.

Values for  $P_e$  measured in 0.02M-KCl + 0.001M-NH<sub>4</sub>Cit are also highly correlated with "A" (Table 12), but are about 30% higher than "A" on acid soils. This suggests that even 0.001M-NH<sub>4</sub>Cit removed phosphate from acid soils with which <sup>32</sup>P ions do not exchange in the greenhouse. Acid reagents (0.3N-HCl; 0.002N-H<sub>2</sub>SO<sub>4</sub>; 0.5N-CH<sub>3</sub>COOH and citric acid) often used in soil analysis also dissolve soil phosphate that does not exchange with <sup>32</sup>P ions in the greenhouse (*Rep. Rothamst. exp. Sta.* for 1958, p. 51). For calcareous soils  $P_e$  values in the presence of citrate are very highly correlated with, but less than, "A"; this is consistent with measurements in the laboratory (Table 1).

The correlations summarised above were obtained with arable soils.  $P_e$  is not always numerically the same value as "A" measured

by growing grass in the greenhouse for 3–4 months if the soils contain either mineral or bone phosphates, which continue to dissolve slowly during a greenhouse experiment, or organic phosphates from plant residues or soil organic matter, which mineralise slowly. The slow release of  $^{31}\text{P}$  (with which  $^{32}\text{P}$  did not exchange during the first few weeks in the greenhouse) from all these materials decreases the specific activity of the later cuts of grass. "A" values calculated in these circumstances may be anomalously high (Mattingly & Widdowson, 1956).

TABLE 13

*Comparison of isotopically exchangeable P values in laboratory and greenhouse experiments and yields of ryegrass in the greenhouse*

Manurial treatment in the field			mg. P/100 g. soil			$P_r/P_e$	g. dry matter
		$A_1$	A	$P_e$	ratio	per pot	
Agdell Rotation, Rothamsted:							
NPK	(f)	...	9.4	10.9	9.1	0.621	3.10
NPK	(c)	...	6.6	7.5	7.8	0.483	1.87
PK	(f)	...	8.7	9.5	9.3	0.619	3.28
PK	(c)	...	5.0	6.4	6.7	0.544	2.02
O	(f)	...	2.7	2.8	3.8	0.547	1.11
O	(c)	...	2.6	2.4	3.5	0.352	0.78
Barnfield, Rothamsted:							
No nitrogen—							
PKNaMg	...	...	19.7	21.5	22.2	0.695	4.20
P	...	...	20.4	23.8	21.8	0.685	4.37
O	...	...	9.4	10.7	11.0	0.628	3.58
Sodium nitrate—							
PKNaMg	...	...	18.2	20.7	20.2	0.679	4.79
P	...	...	17.8	21.0	20.0	0.689	4.42
O	...	...	5.9	7.1	7.5	0.587	2.68
Ammonium sulphate—							
PKNaMg	...	...	32.4	33.9	29.6	0.750	4.65
P	...	...	31.0	36.1	30.1	0.810	4.40
O	...	...	9.3	10.4	12.0	0.585	3.09
Saxmundham, Rotation I:							
P	...	...	11.2	14.4	10.8	0.669	4.19
O	...	...	2.8	3.4	2.8	0.533	1.32
NP	...	...	10.9	13.2	9.3	0.621	4.16
N	...	...	2.6	3.3	2.8	0.473	1.20

## Notes

Agdell Rotation: (f) = fallow rotation; (c) = clover rotation. Manuring 1848–1951; all fertilisers were applied at the following rates per acre once in four years. N = 2 cwt. ammonium sulphate + 18 cwt. rape cake; P = 4 cwt. superphosphate; K = 3 to  $3\frac{1}{2}$  cwt. potassium sulphate. For details of the rotation and soils see Warren (1958).

Barnfield: Rates of manuring/acre/year: N = 4 cwt. ammonium sulphate or 5 cwt. sodium nitrate; P =  $3\frac{1}{2}$  cwt. superphosphate; K =  $4\frac{1}{2}$  cwt. potassium sulphate; Na = 2 cwt. sodium chloride; Mg = 2 cwt. magnesium sulphate.

Saxmundham: Rates of manuring/acre/year: N = 2 cwt. sodium nitrate; P = 2 cwt. superphosphate; K = 1 cwt. potassium chloride. For details of the rotation and soils see Cooke *et al.* (1958).

*Effects of manuring and cropping in the field on "A" and  $P_e$* 

Table 13 gives values for  $P_e$ , the ratio  $P_r/P_e$  and "A" obtained with soils from long-term field experiments at Rothamsted and

Saxmundham Experimental Stations. Yields of ryegrass on these soils in a greenhouse experiment are also given. Values for  $P_r$  and  $P_e$  were obtained by the routine laboratory method (p. 248).  $P_r$  was measured after isotopic exchange for 20 hours and  $P_e$  after 170 hours. Two values are given for "A". The first,  $A_1$ , was calculated from the specific activity of the first cut of grass; the second is the weighted mean value during the experiment (p. 257).

Yields of beans and clover in 1956 and 1957 and phosphate solubility in 0.5M-NaHCO<sub>3</sub> show that soils from the clover rotation on Agdell now supply less phosphate to crops than soils from the fallow rotation (Warren, 1958). The "A" and  $P_e$  values, the  $P_r/P_e$  ratio, and yields in the greenhouse, are also lower on soils from the clover rotation (Table 13).

Yields in the greenhouse,  $P_e$  and  $P_r/P_e$ , are all higher on the *unmanured* soil from Barnfield "No N" plots than on NPK plots from the adjacent Agdell rotation. The application of 4 cwt. superphosphate/acre once in 4 years on Agdell has resulted in a net gain of 8 lb. P/acre/year on the NPK (fallow) and 5 lb. P/acre/year on the NPK (clover) plots (Warren, 1958). This gain has not maintained the total "pool" of isotopically exchangeable phosphate on the more heavily cropped Agdell soils at the present level of the unmanured Barnfield soil. Superphosphate applied at 2 cwt./acre annually at Saxmundham for 60 years, however, has maintained "A" values at higher levels (Table 13) than on Agdell. Yields in the greenhouse on soils from the P and NP treatments at Saxmundham were near the maximum for the experiment.

The supplementary manuring on Barnfield with K, Na and Mg, in the presence of superphosphate, for over 80 years has not affected either  $P_e$  or the ratio  $P_r/P_e$ . Continuous application of ammonium sulphate for 80 years lowered the pH of the soil to about 5.5 by 1955. These plots were limed in 1956 and are now neutral. The "A" and  $P_e$  values on plots receiving P or PKNaMg are 50% higher (Table 13) than the corresponding plots receiving sodium nitrate which have never been acid. This suggests that phosphate was dissolved by ammonium sulphate from particles of chalk and then adsorbed in an isotopically exchangeable form after liming.

#### *Comparison of phosphate removed by ryegrass in the greenhouse with changes in $P_e$*

Preliminary experiments (*Rep. Rothamst. exp. Sta.* for 1957, p. 60) showed that the differences between  $P_e$  values of soils before and after cropping in the greenhouse were *greater* on acid soils and *smaller* on calcareous soils than the total phosphate removed by ryegrass. These conflicting results were probably obtained because  $P_e$  values were measured by exchange in 0.02M-KCl + 0.001M-citrate in which  $P_e$  values are greater than "A" in acid soils and less than "A" in calcareous soils (Table 12).

Table 14 gives more recent results, in which  $P_e$  values were measured in 0.02M-KCl, for soils from two nurseries of the Forestry Commission. At both centres the soils have received superphosphate annually for over 10 years and have been maintained at

different pH values. The Kennington Extension nursery is on an old arable soil (ca. 10% clay) and the Wareham nursery on sand (ca. 1% clay).

TABLE 14

Comparison of phosphate removed by ryegrass with changes in  $P_e$  for soils of different pH from Wareham, Dorset and Kennington, Oxon.

	Kennington (KE27)					Wareham (W27)					Mean of all soils
	4.3	4.8	5.3	6.2	7.0	3.8	4.8	5.6	6.5	6.8	
Approximate pH ...	4.3	4.8	5.3	6.2	7.0	3.8	4.8	5.6	6.5	6.8	—
$P_e$ (before cropping), mg. P/100 g. ...	14.4	13.6	15.4	17.6	18.3	12.6	13.6	16.1	19.9	17.1	15.9
$P_e$ (after cropping), mg. P/100 g. ...	4.0	3.1	3.2	3.4	3.3	2.0	2.0	2.6	4.4	4.2	3.2
Difference, mg. P/100 g.	10.4	10.5	12.2	14.2	15.0	10.6	11.6	13.5	15.5	12.9	12.7
P removed by ryegrass, mg. P/100 g. ...	11.6	12.6	12.4	14.2	14.4	10.0	12.6	15.4	17.4	16.2	13.7

The decrease in  $P_e$  by cropping on both soils is almost equal to the phosphate removed by the ryegrass. This confirms that the laboratory method used here to measure  $P_e$  estimates quantitatively the "pool" of isotopically exchangeable phosphate in soil taken up by ryegrass over the whole pH range from 3.8 to 7.0.

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## THE HISTORY AND WORK OF THE SOIL SURVEY

BY

A. MUIR & D. A. OSMOND

Mankind must have taken an interest in soil from the time when "Adam delved and Eve span", but the first record appears to date from about the year 2210 B.C. when "Da Yu, the first emperor of the Hsia dynasty, took up the study of the soils of nine territories and classified them according to their colour, texture, geographical features and productivity for the purposes of evaluation and assessment of land taxes" (Tang, 1935). However, information about the distribution of the different kinds of soils was not collected systematically in other parts of the world until late in the 18th century. In his Presidential Address to the III International Congress of Soil Science, Sir John Russell said: "One of the striking services that soil science has rendered in recent years has been in surveying soils of the different countries and in the preparation of maps on which any desired part of the information can be represented. This is now recognised as an essential preliminary to all agricultural developments, reclamations and irrigation schemes, and it forms an integral part of any organised development of agriculture such as is now being carried out in many countries of the world. To start on important agricultural development without a preliminary soil survey is to run serious risk of disaster." The importance of the last sentence cannot be overstressed, and it should be borne in mind by all in charge of planning agricultural developments. That the warning has been heeded is apparent from the number of large enterprises in all parts of the world in which one of the first demands is for a soil survey to provide basic background information.

Although in 1665 a committee of the Royal Society circulated a questionnaire to numerous gentlemen asking for information about the kinds of soils in various countries, little was done to collect it and it was not recorded on maps. Later, in 1683, Dr Martin Lister presented to the Society "An Ingenious proposal for a new sort of Maps of Countries, etc.", which was intended to include both rocks and soils; but whether "soils" was used in the more modern sense is not clear—from Lister's description it appears that reference was made only to the top-soil. Not until the end of the 18th century were the first attempts made to prepare soil maps, when the authors of the General Views on Agriculture were requested to do so, and many of the Reports to the Board of Agriculture include maps of the distribution of the various soils in the country. Both top-soil and sub-soil were sometimes described; but land use or superficial geological deposits were often represented rather than soils.

By the 19th century geological maps had been produced and scientists interested in soil naturally turned to them, thinking that, if soil arose from the weathering of rock, then the distribution of soils

and rocks should be closely related—a belief that is valid only in part. It is not surprising, therefore, that early workers in soil survey, such as Gilchrist (1907), Hall and Russell (1911) and Newman (1912), should have been strongly biased towards using geological maps as the basis for soil maps. It is interesting to notice that these attempts at making soil maps were promoted by scientists working in southern England, where the areas surveyed were only thinly, if at all, covered by glacial drift and where it was feasible in many places to regard the underlying rock as the parent material of the soil. Consequently terms such as “Chalk soil”, “Gault soils”, “Lias soils” and so forth were commonly used and still usefully persist, though their sense may have been refined. However, when one of the best-known workers in British soil science, G. W. Robinson, made a soil survey in Shropshire he began to realise that the intimate relation between solid rock and soil was not so applicable in country that had been glaciated in past eras. This was more forcibly impressed on him when he was appointed Advisory Chemist in North Wales and initiated a soil survey of Wales.

In the course of innumerable visits to farmers, advisory chemists became aware of the many problems of plant growth which could not be entirely solved by applications of fertilisers but which were related to more fundamental and relatively unchangeable soil properties associated with the whole soil profile. They therefore began seriously to canvass the possibility of making maps of the soil as such. Maps were being made in the U.S.A. in which soil drainage and the nature of the subsoil were included in the description of what was called the soil series, but it was not until 1920, after a visit to the U.S.A. by W. G. Ogg, and later G. W. Robinson, that the importance of the soil profile and its morphology was appreciated in Britain and that attention was directed to the achievements of the Russian pedologists.

In Great Britain a sub-committee of the Development Commission considered, in 1919, the possibility of making soil maps which would, however, mainly be improved versions of the existing geological maps. From the accounts by Ogg and Robinson of the work in the U.S.A., it became clear that an independent body should undertake soil survey, though officials were reluctant to set up an organised survey. However, the Ministry of Agriculture agreed that certain Advisory Chemists should be allowed assistants to make soil maps. Incidentally, about this time G. W. Robinson introduced into the English language the words “pedology” to describe the scientific study of soils and “pedogenic” to describe the processes of soil formation and metamorphism.

In 1926 the American method of mapping soil series was demonstrated by G. Newlands and W. Dow at the first Soil Survey Conference, held at Harper Adams Agricultural College, when the first soil series map made in England was made of the College farm. After this it was decided to adopt the U.S. system of series and types (texture classes) for use in Great Britain, and at subsequent conferences and field meetings in England and Scotland methods were standardised for surveying and recording the information on maps.

In 1922 the Ministry's Conference of Advisory Chemists recommended that attempts to “correlate fruit culture with soil types

should be made in East Anglia and in the West Midland counties", and during the next 20 years the results of several such fruit-soil surveys were published. As much of the work was started before 1926, soils were not described by series, and it was difficult to reconcile some of this older work with the newer ideas prevailing when it came to be published. The report of the fruit-soil survey in East Anglia (Wright & Ward, 1929) was published with maps showing the distribution of soil types (classes), and an attempt was made to relate plant growth to soil type. The second survey, in the West Midlands (Wallace *et al.*, 1931), described what are now recognised as soil series and drew attention to the inherent properties of the soil profile conducive to "good" or "bad" growth of fruit trees as evidenced by measurements of trees of comparable age and variety grown under similar systems of management. In addition, nutritional disorders were investigated and remedial measures suggested. Field trials and laboratory research were initiated on problems arising out of the survey.

These results and many others important to fruit growers and market gardeners were substantiated and extended by later reports on other fruit-growing areas, by Ward (1933) on West Cambridgeshire, Bane & Gethin-Jones (1934) on the Lower Greensand in Kent and Bagenall & Furneaux (1949) on the Hastings Beds, also in Kent. The last report contained the comment that "So local was the distribution of these soil series that it proved difficult to find a single orchard or plantation that was perfectly uniform in soil throughout", and although series were described so that they could be identified in the field, no soil maps were made. In the fruit-growing district of the Vale of Evesham (Osmond *et al.*, 1949), similar plants were measured to relate the behaviour of fruit trees and horticultural crops with soil properties; the distribution of the series was recorded on maps. Together these surveys covered the largest fruit-growing districts and, besides obtaining information of use in solving some fruit-growing problems, they provided a sound basis for giving advice on planting new sites. The American series system was also used to describe the soils in the Vale of the White Horse (Kay, 1934) and later the strawberry district in South Hampshire (Kay, 1939). In her survey of the Vale of the White Horse Kay showed that Brenchley's rough correlation of weeds with soils could be made more definite and that certain weeds were associated with distinct soil series.

Other surveys, not all for their immediate practical value, were made in various places. G. W. Robinson, who founded a "school" of pedology in Bangor, made, with his staff, extensive soil surveys in North Wales. W. M. Davies and his colleagues began a survey around Harper Adams Agricultural College, following the demonstration of series mapping by Newlands and Dow; the work was eventually published in 1954 as one of the first of the memoirs of the Soil Survey of Great Britain. In the late 1920s W. G. Ogg began a soil survey of East Lothian after completing the mapping of the Edinburgh and East of Scotland College of Agriculture farm, and G. Newlands mapped parts of Aberdeenshire. A survey on the basis of soil texture was made of the district around Ayr by McArthur *et al.* (1932). After the Macaulay Institute was founded in 1930,

soil survey in Scotland was done mainly from there. The early interest was in classification and soil genesis, but some forest areas and, later, agricultural land were also surveyed (Muir, 1934, 1935; Muir & Fraser, 1940; Glentworth, 1944). During the progress of these various surveys many problems arose in the actual methods of surveying, as well as of classification of soils; these were discussed at the annual Field Meetings held in different parts of Britain, during which parties of surveyors mapped the same area and compared the results.

With surveys extending, there was evident danger that the same soil might be differently named by surveyors in different parts of the country; the identification of the parent material (when a drift deposit and not a solid rock) was a source of possible trouble, and there was obvious need for a standard system of classification. A Soils Correlation Committee was therefore set up to consider these matters, and members made tours in England and Wales in 1930 and 1935 and in Scotland in 1932 to examine soil series being mapped.

The Soils Correlation Committee was replaced in 1936 by the Soil Executive Committee with similar functions, and in 1939 it was decided to set up the Soil Survey of England and Wales with Prof. G. W. Robinson as Director; a close connexion was maintained with the soil survey in Scotland conducted from the Macaulay Institute. A small committee, formed in 1938 to discuss colouring soil maps, prepared a working classification of soils as then known. This, together with a classification of parent materials prepared by the Correlation Committee, was included in a *Soil Survey Field Handbook* by G. R. Clarke which provided standardised methods for describing soil profiles, classifying soils and colouring maps that remained in use for many years. However, recent advances, both at home and abroad, in the technique of describing and mapping soils showed deficiencies in the *Handbook* which has now been revised and a new edition published (1960).

During the War soil survey almost ceased, but the knowledge acquired was of great use in the recommendations made by committees for ploughing up old grassland and allocating the limited amounts of fertilisers. The survey officers in effect became advisory officers, and when the National Agricultural Advisory Service was formed after the War, some joined the new organisation. The headquarters of the Soil Survey of England and Wales was transferred to Rothamsted Experimental Station, and A. Muir was appointed Head of the Survey in 1946. In Scotland the Macaulay Institute continued to be the headquarters of the Scottish Soil Survey. To guide the Surveys, a Soil Survey Research Board was set up by the Agricultural Research Council.

Until recently, mapping in England and Wales was done at 1 : 63360, and four memoirs (Crompton & Osmond, 1954; Avery, 1955; Roberts, 1958; Ball, 1960) with soil series maps have so far been published and a map of the soils of the Pwllheli district without a memoir (1958); several more maps and memoirs are nearly completed. Most of the surveys are done to obtain knowledge of the distribution of soils, but the Glastonbury district was surveyed because of the teart disorder of cattle in Somerset. Although the

survey did not solve the problem, it directed attention to the prevalence of the trouble on particular soils associated with the Lower Lias formation, which were later shown to promote unusually high contents of molybdenum in the pasture plants. Since then excess or deficiency of this element has been shown to be important in animal and crop husbandry in many parts of the world. Deficiencies of other minor elements have come to light and their correlation with soil series shown in other parts of Great Britain. As might be expected, some of these minor-element troubles in this country depend on the kind of rock from which the soils are derived. Thus, cobalt deficiency is linked with certain areas of granite in Devon and Cornwall and with some areas of Old Red Sandstone in the north of Scotland. Nevertheless, considerable changes in minor-element content can be produced in soils by differences in drainage.

In Scotland the survey is made mainly at 1 : 25,000 and four memoirs (Glentworth, 1954; Muir, 1956; Mitchell & Jarvis, 1956; Ragg, 1960) with maps have been published; others are being prepared. Mainly at the instigation of the National Agricultural Advisory Service, reconnaissance mapping at 1 : 25,000 has been adopted in England and Wales since 1959, and a considerable area has been mapped on this basis; the surveyors are stationed at the regional headquarters of the National Agricultural Advisory Service, with mutual benefit to both.

Many official bodies are now taking heed of Sir John Russell's words quoted above, and requests for surveys of particular areas are numerous. All the Experimental Husbandry Farms, sites of many field experiments of the National Agricultural Advisory Service and several county farm institutes have been surveyed, and it is hoped that the relation between crop, management and soil will become better established than now and so allow better advice to be given and yields better predicted. Similar surveys have been made for the Forestry Commission, and a map is being made of the soils in Thetford Forest—the largest in England.

It would be tedious to detail all the *ad hoc* surveys (see *Annual Reports*) undertaken, but some of the more interesting deserve mention. In 1950 a preliminary survey, which was later extended, was made of the Wentlloog and Caldicot Levels (Glamorgan) where 26,000 acres were mapped to determine the extent of poorly drained soils on which productivity is limited although their inherent fertility is high. In the same year a survey was initiated of some of the "moss" lands in Lancashire; the information provided on the differing kinds of peat soils, their thickness, the nature of the underlying mineral soil and their agricultural potential aroused so much interest that the survey was extended; by 1957 35,000 acres were mapped, and the report was incorporated in the recommendations made about their use and drainage. One of the problems of opencast iron- and coal-mining is to restore the sites to agricultural use afterwards, and several surveys have been made at different times in this connexion; the rate at which large agriculturally intractable blocks of limestone weather and the rate of soil formation on these highly disturbed, heterogeneous materials has also been studied. Deposits of Shirdley Hill Sand in Lancashire subjected to certain pedological processes yield a quartz sand highly suitable for

glass-making. In waterlogged soils, particularly under a peaty organic covering, iron oxides are strongly reduced, and the reduced iron readily diffuses out of the upper layers, leaving a strongly bleached residue. When this occurs in the Shirdley Hill Sands, it leaves an excellent glass sand. Thus the soils when classified genetically fall into groups that fairly closely represent glass-sand quality. As much of the deposit was in an area proposed for a new town, a survey was made in 1958-59 to determine the extent of the glass-sand; its distribution and quality were mapped, and as a result a sequence of building was arranged so that this valuable resource should not become unusable as happened in a district built over earlier.

Much horticultural land is level and well-drained, so it is often coveted for building sites, and surveys have been made to delimit its extent, notably in Sussex and Guernsey. The whole of Guernsey was surveyed, and in Sussex the survey is being extended to cover the whole coastal district. In connexion with the proposed routes of new roads, by-passes, etc., in various parts of England, soil profiles were described at frequent intervals and samples supplied to the Road Research Laboratories for investigation. Surveys have been made of several large areas, proposed as the sites of new towns, where there is a possibility of the loss of much high-class agricultural land. This use of survey information dates from the close of the Second World War when land classification maps were made of Herefordshire, Worcestershire, Shropshire, Warwickshire and the environs of Birmingham for the West Midland Group on Post-War Reconstruction and Planning (West Midland Group, 1946, 1947, 1948). Specifications of site and soil were drawn up by a committee, on which the Soil Survey was represented, to enable three "qualities" to be differentiated, and maps and reports were presented on this basis. A similar survey was made later in Somerset, Gloucestershire and Wiltshire for the Reconstruction Research Group (1947) of Bristol University, and the method is now being used by the Ministry of Agriculture, Fisheries and Food in surveys of potential horticultural land. Maps and reports have also been made more recently by the Soil Survey on similar projects for new towns in the north of England and in connexion with post-war planning in the Midland Valley of Scotland.

Although these *ad hoc* surveys mean there is less time for "routine" surveys, the two do not always conflict. Sometimes the information needed exists in the routine survey and needs only to be put in an appropriate form for immediate use; at others the request leads to an extension of the survey, or, information from an *ad hoc* survey becomes of use when it is later decided to make a routine survey of that particular district.

In addition to preparing maps and memoirs and reports on specific areas, the Survey publishes an *Annual Report* (1950-59) describing the year's work, with brief descriptions of new soil series and *ad hoc* surveys.

A soil map of Great Britain (1 : 2,500,000), made from a reconnaissance survey by the staff at 1 : 625,000, is incorporated in the soil map of Europe prepared by the Food and Agriculture Organisation of the United Nations, and officers of the Survey take part in

international discussions concerned with soil classification and the preparation of a soil map of the world.

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