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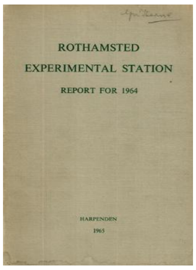
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SPECIAL REVIEWS

THE POTATO VARIETY KING EDWARD VII AND PARACRINKLE VIRUS

F. C. BAWDEN and B. KASSANIS

The story of potato paracrinkle virus is worth telling for several reasons in addition to its intrinsic interest. It illustrates, as well as any other, both the extent to which knowledge about the behaviour of viruses has grown during the past 30 or so years and the unsuspected complexity of this behaviour. Perhaps better than most, it shows how practical problems can produce subjects for academic research and how in turn this research can benefit practice. It also provides a valuable cautionary tale, showing that science is not free from its myths, and that, although these mainly arise when speculation outstrips facts, what is factual is often far from sure. Most of the features of paracrinkle virus generally accepted in the 1930s as established have since been proved to be untrue.

The discovery of paracrinkle virus. Paracrinkle virus was discovered at the Potato Virus Research Station, Cambridge, where in the late 1920s Dr. R. N. Salaman was engaged in the practical task of increasing the yield of the commonly grown potato varieties. His aim was to replace the diseased stocks then in use with healthy ones and, as a first step, he was seeking virus-free plants, whose progeny could then be bulked and put into commerce. His method was to select vigorous plants growing in the best potato "seed-growing" districts, take tubers from them to plant in glasshouses at Cambridge, where the foliage was critically examined for symptoms and the plants were further tested by grafting scions from them on to indicator potato varieties and inoculating their sap to potato and to other species, such as tobacco (*Nicotiana tabacum*) and *Datura stramonium*.

The common virus diseases then recognised were leaf roll, crinkle, severe mosaic and mild mosaic, of which crinkle is caused by the joint action of two viruses later identified and named potato A and X, severe mosaic by virus Y and mild mosaic by virus X. It proved easier to get plants of the variety King Edward VII free from these viruses than of most other varieties, because these viruses all produce evident symptoms in King Edward, and virus X, which was then almost universal in many varieties, kills King Edward plants. Nevertheless, it proved impossible to find a virus-free King Edward plant, for scions from every one tested, however vigorous, when grafted to the variety Arran Victory caused a crippling disease, which Salaman and Le Pelley (1930) called paracrinkle. The symptoms, especially of plants in their second and later years of infection, differed from any previously described. Although individual plants differed somewhat in the severity of their reaction, a fact that Salaman (1932) wrongly attrituted to a variable association between paracrinkle virus and virus Y, characteristically the young shoots of grafted Arran

Victory plants first developed bright yellow blotches on their upper leaves, which also became severely waved and crinkled. Plants grown from the tubers of infected plants were mostly miserable, reaching only a few inches high, with shortened internodes and grossly deformed leaves, a condition aptly described as curly dwarf. Some varieties other than Arran Victory also developed severe symptoms when infected by grafting, but some were little affected, and the variety President looked normal.

Salaman and Le Pelley failed to infect other potato varieties or other plant species by inoculating them with sap from King Edward and also failed to transmit paracrinkle virus with the aphid *Myzus persicae*. Other features also seemed to suggest that the virus had no vector. For example, although King Edward was the second most widely grown potato variety in England, and so each year there were vast numbers of plants providing potential sources of infection, paracrinkle was never reported occurring naturally in plants of varieties that show the disease when infected by grafting; also, with King Edward and Arran Victory planted alternately in rows at Cambridge, tubers saved from the Arran Victory plants, although often infected with leaf roll or virus Y, were never infected with paracrinkle virus.

Various other workers confirmed that grafting Arran Victory with scions from King Edward plants always produced paracrinkle, and it became increasingly likely that the virus was present in the whole clone, although this was obviously impossible to prove. These other workers discovered nothing to conflict with the conclusion of Salaman and Le Pelley that King Edward was the "perfect carrier" and unharmed by the virus, which seemed distinct from any other and to be only of academic interest because it had no natural method of spread into plants it could harm.

Paracrinkle virus and plasmagenes. Little research was done on paracrinkle virus for some years, and perhaps little more would have been done had the virus not become a prime subject for speculation in discussions on the origin of viruses. The isolation in the 1930s of several viruses as nucleoproteins suggested that their nearest analogues might be normal nucleoproteins rather than the pathogenic micro-organisms to which most workers had previously related them. In turn this suggested a possible origin of viruses either in faulty synthesis during normal nucleoprotein metabolism or by the accident that a nucleoprotein of one type of organism might get introduced into another where it could multiply and be pathogenic.

Bawden (1939) pointed out that there seemed two equally plausible explanations of what was then accepted knowledge about paracrinkle virus: (1) that the original King Edward seedling became infected with a virus that had since lost its natural method of spread and been perpetuated only by the vegetative propagation of its host; (2) that it was a normal component of King Edward transmissible by grafting to other potatoes, in some of which it was pathogenic. He stressed the uncertainties of any interpretation and that nothing was known about the early history of the variety, but the idea that the virus was intrinsic to King Edward was accepted by some writers as though it were established (Darlington, 1944; 1949; Plank, 1948). Paracrinkle virus was advanced as evidence both that viruses derive from plasmagenes, the postulated entities responsible for characters inherited through the cytoplasm, and that a cell protein beneficial in one genotype could be destructive in another. Darlington (1949) separated it off from other viruses in a group he called "provirus", distinguished by being transmissible only by grafting and becoming a virus when introduced by grafting into another genotype.

As the parents of the King Edward variety are unknown, the same cross could not be repeated to see whether it would re-create the virus. (According to Salaman (1926) the original seedling was bred by a gardener in Northumberland, who called it Fellside Hero, and after passing from him to a farmer in Snaith, Yorkshire, it reached J. Butler, who multiplied the stock and marketed it in 1902 under the name King Edward VII.) Also, King Edward plants rarely flower, so the inheritance of the virus is difficult to study, but differences from the expected behaviour of a plasmagene were evident in the fact that none of 22 seedlings from seed set by King Edward pollinated from the variety Flourball contained paracrinkle virus (Carson, Howard, Markham and Smith, 1944).

The postulate that paracrinkle virus arises as a consequence of grafting was invalidated when paracrinkle was produced by inoculating Arran Victory plants with sap from King Edward. Salaman and Le Pelley (1930) failed to do this because their method of inoculation (scratching leaves with a needle through a drop of sap) was inadequate. When leaves of Arran Victory plants were dusted with a diatomaceous earth or carborundum powder and then rubbed with sap from King Edward some developed paracrinkle, and the proportion that did increased when the Arran Victory plants were kept in darkness for a few days before they were inoculated (Bawden, Kassanis and Nixon, 1950). These methods of inoculation and treatment of plants also increase the ease with which many other viruses are transmitted by inoculation. Had Salaman and Le Pelley inoculated sap to tomato (*Lycopersicum esculentum*) instead of to tobacco and *Datura stramonium*, they might well have discovered its transmissibility, for whereas the two plants they used are immune to the virus, tomato is much more susceptible to infection than potato, and their method is adequate to infect this host. However, they might still have failed to do so, for infected tomato plants show no evident effects on their foliage or growth, and the infection would have remained undetected unless they had grafted scions from the tomato plants to Arran Victory plants.

The transmission by inoculation to tomato gave the first information about the particles of paracrinkle virus and allowed some of its properties to be studied. Infected tomato plants contained specific particles about 10 mμ wide and of various lengths, resembling some other viruses both morphologically and in their susceptibility to destruction by heating. Sap from King Edward plants and plants of Arran Victory with paracrinkle, whether infected by grafting or inoculation, also contained elongated particles, but the identity of these was uncertain, because supposedly virus-free plants of Arran Victory and of some other potato varieties, including some recently bred seedlings, also contained somewhat similar elongated particles. Some of these were cellulose fibres, but later work makes it probable that others were potato virus S, then unknown but soon to be discovered and, ironically, to prove to be related to paracrinkle virus.

Virus-free King Edward. The transmission of paracrinkle virus by sap inoculation and the knowledge about its morphology and properties brought it into line with other viruses and, by abolishing its seeming uniqueness, also abolished the need to assume any different relationship between it and the King Edward potato than between any other virus and a tolerant vegetatively propagated host. The idea that paracrinkle virus derives from a plasmagene or other endogenous component of King Edward was finally disproved by the demonstration that not every cell contains it and that, as with some other viruses, the stem apical meristems are not infected. Applying this knowledge and culturing apical meristems on nutrient medium produced a virus-free clone of King Edward (Kassanis, 1957), something heat therapy had failed to do (Bawden, Kassanis and Nixon, 1950). Most apical meristems did not differentiate and yielded only callus tissue, but one grew into a plantlet, which was virus-free. When large enough to handle, it was grafted on to a tomato plant, and after it was well established as a scion, cuttings were taken from it and established on their own roots. The resulting plants grew vigorously, producing enormous haulms, but when harvested in October none had any tubers, presumably because light and temperature conditions in the glasshouse were unsuitable for tuber formation. Further cuttings were taken, which were rooted and grown in controlled daylengths and temperatures, as were similar cuttings from the parent infected clone (Kassanis and Schwabe, 1961). Both clones then produced tubers, but comparisons of the plants showed that King Edward is not the perfect carrier of paracrinkle virus it had been assumed to be. Plants from the virus-free clone were more vigorous than the others, had more leaves and a larger leaf area, and produced more total dry weight and weight of tubers. Effects of the virus were also obvious when the two clones were grown side by side in the open, for plants from virus-free tubers were not only more uniform but their darker green, flatter and larger leaves covered the ground more completely than the foliage of plants from infected tubers.

The virus-free clone was multiplied under glass at Rothamsted from 1955 to 1957, when several hundred small tubers were given to the National Institute of Agricultural Botany to propagate in the field and conduct yield trials. The results of these trials, summarised in the table kindly provided by Mr. J. C. Cullen, show that the more vigorous haulm of the virus-free clone is reflected in an increased weight of tubers. The average of about 10% more yield than from the best obtainable commercial stocks comes from an increased number of tubers and not increase in size of tubers. A commercially desirable feature of the virus-free clone is that its tubers are more uniform in size, which is shown in the table by the fact that, although the weight of tubers passing over a 2-in. riddle is not much more than with the commercial stocks, the weight passing over a $1\frac{5}{8}$-in. riddle is.

The virus-free clone has now been extensively propagated and is likely soon to supplant other stocks, which means that, with no extra effort by growers, King Edward potato crops are likely to yield around 10% more in the future than in the past. The virus-free clone has produced variants with wholly red tubers, and so a virus-free line of Red King will also be able to take the place of the current infected ones.

Yields of tubers from the virus-free clone and seven commercial stocks of King Edward in trials by the National Institute of Agricultural Botany

Yield in Tons/acre

K = Paracrinkle Free Stocks A–G = 7 Commercial Stocks

	K	A	B	C	D	E	F	G
Total Yield:								
1959 (4 centres)	13·9	12·8	12·4	12·9	12·5	12·2	12·9	12·6
1960 (9 centres)	13·8	12·0	12·7	12·1	12·3	12·6	12·4	13·0
1961 (9 centres)	16·8	15·6	15·7	15·1	15·7	15·4	16·0	15·6
1962 (9 centres) *	16·7	15·2	15·6	15·5	15·6	15·6	15·7	15·2
1959–62	15·3	13·9	14·1	13·9	14·0	14·0	14·3	14·1
Yield Over 2 in.:								
1959 (4 centres)	5·0	5·5	5·6	5·7	5·4	5·3	5·4	5·6
1960 (9 centres)	7·4	7·4	7·5	7·1	7·1	7·5	7·1	8·1
1961 (7 centres)	12·3	11·9	12·1	11·6	11·6	11·4	12·0	11·4
1962 (9 centres) *	10·9	10·8	10·8	10·7	10·0	10·8	10·6	10·3
1959–62	8·9	8·9	9·0	8·8	8·5	8·8	8·8	8·9
Yield Over 1$\frac{5}{8}$ in.:								
1961 (8 centres)	14·9	13·7	14·2	13·4	13·9	13·7	14·4	14·0
1962 (9 centres) *	15·0	14·1	14·3	14·3	14·1	14·2	14·4	13·7
1961–62	15·0	13·9	14·3	13·9	14·0	14·0	14·4	13·9

* Stock C, D, E and F omitted at 1 centre.

Removing paracrinkle virus seems to have had no effect on the qualities of King Edward potatoes, except in vigour of its haulm and yield of tubers. Nor has it affected reaction to or susceptibility to infection by other viruses; in particular, the virus-free clone still retains its hypersensitivity to infection by virus X, and so it is unlikely that field crops will become infected with this virus.

Relationship with other viruses. Evidence relevant to the evolution of paracrinkle virus and suggesting that King Edward contracted it by infection with a virus carried by aphids came from an unexpected source, work on viruses infecting carnation (Kassanis, 1954, 1955). One of these, the aphid-transmitted latent virus, had particles indistinguishable from paracrinkle virus, and antiserum prepared against it precipitated specifically with sap from infected King Edward plants. This suggested a relationship between paracrinkle and an aphid-transmitted virus, but was not conclusive, because the antiserum also precipitated specifically with sap from seemingly healthy plants of other potato varieties, including Arran Victory. This fact became explicable when it was shown that these other varieties were infected by potato virus S, which was discovered by Ouboter (1951) while making serological tests during attempts to prepare an antiserum against potato virus A, and which she later found was prevalent in symptomless plants of many potato varieties grown in Holland. It has since been found to be widespread and shown to be serologically related to paracrinkle virus, which it accompanies in many King Edward plants (Kassanis, 1956, 1961; Rozendaal and van Slogteren, 1958; Bagnall, Wetter and Larson, 1959).

As all known stocks of Arran Victory were infected with virus S, the question arose as to whether the symptoms of plants infected from King Edward are caused by paracrinkle virus or by it acting jointly with virus S. To answer this, apical meristem culture was used to produce a clone of Arran Victory free from virus S (Kassanis, 1957), and this was infected with paracrinkle virus alone and together with virus S. The presence of virus S did not affect symptoms, and variability in their severity seems to depend solely on the virulence of the strain of paracrinkle virus. Indeed, the concept of the perfect carrier for long wrongly applied to King Edward and paracrinkle virus can perhaps be more appropriately applied to Arran Victory and virus S, for the virus-free clone is not noticeably more vigorous than its infected parent and has yielded little better in field trials. Also, it may be that King Edward is the perfect carrier of virus S rather than paracrinkle, for stocks infected with both viruses are not noticeably different from those infected with paracrinkle virus alone. However, the effect of infection on yield by virus S alone has not been tested.

Most pairs of serologically related viruses interfere with each other's multiplication, and plants fully infected with one resist invasion by the other, but this is not so with virus S and paracrinkle, each of which multiplies to much the same extent when together in plants as when alone. The lack of interference probably reflects the remoteness of the relationship between the two, shown by the fact that antiserum made against one may have a precipitation end-point exceeding $\frac{1}{1000}$ when titrated against that one, but fail when diluted beyond $\frac{1}{16}$ to precipitate the other. Kassanis (1961) suggested that viruses so slightly related serologically should be distinguished from one another by being called sero-types rather than strains, which should be restricted to those with closely similar antigenic behaviour. Accepting this suggestion, paracrinkle virus, potato virus S and carnation latent virus become sero-types of one virus, as also does chrysanthemum virus B, the latest to be shown to be remotely related to paracrinkle virus (Hakkaart, van Slogteren and Neeltje, 1962). However, in addition to virus S, paracrinkle is related to potato virus M, which causes leaf rolling mosaic and is so antigenically similar to paracrinkle that these two must be regarded as strains (Bagnall, Wetter and Larson, 1959). Leaf rolling mosaic is a common disease in North America, where it was described in detail before paracrinkle was discovered (Schultz and Folsom, 1923), so it takes precedence in nomenclature. Hence, paracrinkle not only ceases to be a name for an individual virus, but even of a distinctive sero-type, and remains only to identify a minor variant of the sero-type leaf rolling mosaic virus.

Transmission by *Myzus persicae*. The discovery that paracrinkle and the aphid-transmitted carnation latent virus are related raised doubts about the validity of the conclusion that paracrinkle virus is not transmitted by *M. persicae*, but first tests seemed to confirm the conclusion, for *M. persicae* failed to transmit the Rothamsted strain of paracrinkle virus in conditions in which it consistently transmitted the carnation virus (Kassanis, 1956). However, with the discovery that aphids also transmit the closely related leaf rolling mosaic virus (Rozendaal and van Slogteren, 1958; Wetter and Völk, 1960), further tests were made using King Edward plants from different commercial stocks as virus sources, and these showed that paracrinkle virus exists in strains that differ in their transmissibility by *M. persicae*, with some being transmitted readily and others only rarely, if ever. Thus, in conditions in which the Rothamsted strain was not transmitted to any of the test plants, three other strains were transmitted to more than half, to a third and to a tenth, respectively. (Kassanis, 1961)

After this knowledge it was not surprising to find that in some of the field trials conducted by the National Institute of Agricultural Botany, where plots of the virus-free clone were surrounded by plots of infected King Edward, a proportion of the initially virus-free plants became infected. The proportion differed greatly in different places and was most in districts where the aphid-transmitted leaf roll and Y viruses also spread most extensively. There have been very few infections in the crops grown in the recognised seed-growing areas, so the clone is unlikely to become reinfected, but it will clearly be necessary to test nuclear stocks regularly to ensure that they are still free from infection.

As no vector for potato virus S has yet been identified, it is of some interest that in the yield trials where the virus-free clone of King Edward became infected with paracrinkle virus it also became infected with virus S. Again there is the obvious possibility that virus S also exists in strains only some of which are aphid-transmitted and that glasshouse tests yet made have been only with strains not so transmitted. However, another possibility is that virus S is transmitted by aphids only when it occurs together in a plant also infected with an aphid-transmitted strain of paracrinkle virus, for several other viruses are known that are not aphid-transmitted when alone but become so in the presence of another virus that is. The early failures to transmit paracrinkle virus probably reflect the fact they were all made from a stock of King Edward infected with a strain not aphid-transmitted, which could also explain the lack of transmission when King Edward and Arran Victory plants were grown adjacent in the open, but an additional factor may be that Arran Victory plants, although they react so severely when infected, are much more resistant to infection by aphids than King Edward.

The surveys of commercial stocks of King Edward recently made show that most do contain aphid-transmitted strains of paracrinkle virus. There are various reasons for paracrinkle not being a prevalent disease in the United Kingdom despite the many sources of infection. First, varieties like Arran Victory that show severe symptoms when infected are rarely grown near to King Edward in districts where aphid-transmitted viruses spread readily, and it is even rarer for seed of such varieties to be saved in such districts. Secondly, aphids can become infective by feeding only briefly on King Edward, but they also soon lose their infectivity; this behaviour favours spread between plants within a crop rather than between separated crops, for few aphids will carry the virus over long distances. Thirdly, except for King Edward, the varieties commonly grown in the

United Kingdom may be resistant to infection. The occurrence in North America of leaf rolling mosaic shows that in other conditions paracrinkle-like viruses can spread in field crops and cause damaging diseases. Aphids are more numerous there than in most parts of the United Kingdom, and there is some evidence that their virus strains are also more readily transmitted by aphids; also, their varieties may be more susceptible to infection. Whatever the explanation for spread in North America, the knowledge that paracrinkle and leaf rolling mosaic are related viruses emphasises the hazards to other varieties from growing infected stocks of King Edward, and provides an additional reason to the extra yield for replacing these stocks by the virus-free clone.

Surveying commercial stocks produced several other results. It showed that paracrinkle virus exists in strains of different virulence towards Arran Victory and that some King Edward plants contain strains causing a disease closely resembling leaf rolling mosaic; that most plants of King Edward are infected with potato virus S in addition to paracrinkle, and that not all plants have paracrinkle virus, for one was found infected with S alone. (Kassanis, 1961)

The origin of paracrinkle virus. From long being thought to be unique, the status of paracrinkle virus has steadily diminished, to become only one of many strains and sero-types in a group whose members differ in their host ranges, their virulence towards different species or varieties of plants, their transmissibility by aphids and, no doubt, in many other ways still to be discovered. What evolutionary significance to place on present knowledge can only be guessed at. Tracing evolutionary courses is difficult with organisms that leave fossil records, mutate rarely and have long generation times, and is impossible with viruses. However, it is plausible to assume that this group of viruses with now very dissimilar host ranges had a common origin in an aphid-transmitted virus that had a much wider host range than any of the existing members of the group yet studied. From what is known about the behaviour of other viruses, it is to be expected that, having entered plants as different as potato, carnation and chrysanthemum, the virus might change in different ways, for each host would preferentially favour any chance variant that multiplies more rapidly in it than the one that initiated the infection. Vegetatively propagated plants once infected remain so for as many generations as their progeny remains in being, and so provide ample time not only for mutants to be produced but also for them to be selected and become dominant. Not that any great length of time may be needed, for changing host plants is sometimes all that is needed to select forms differing by enough to justify distinguishing as sero-types, as when tobacco mosaic virus infects leguminous plants systemically (Bawden, 1958). Loss of transmissibility by aphids is not surprising, for the survival of a virus in a vegetatively propagated plant is independent of this property, which several other viruses have been found to lose when selection for it was not operating. The main thing that will be detrimental to the survival of a virus in clonal varieties of cultivated plants is virulence towards those varieties, for when selecting plants for propagation growers will reject those that are obviously diseased; no

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other explanation is needed for the fact that paracrinkle and the other viruses to which it is related are now prevalent mainly in varieties that are only little harmed by infection.

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ELECTRICAL CHARGES ON CLAY

G. H. CASHEN

A review by R. K. Schofield in the *Rothamsted Report* for 1947 gave a concise account of several new ideas that he had put into the subject. Since then the ideas have been tested at Rothamsted and elsewhere on a scale too big to summarise in a short article: the present review is restricted to work done here, or at least recognisably started here.

Clay lattices have a permanent negative charge because cations of the ideal structure are replaced by others of lower valency, and electrical neutrality is maintained by cations held outside the lattice. These are the exchangeable cations, important in many aspects of soil fertility and soil management. The edges of the clay crystals are not inert, and study of reactions at the edge faces is a necessary part of the study of the clays, but it is the nature and behaviour of the exchangeable cations that determine the suitability of soils for successful plant growth.

When clays are immersed in water or dilute salt solutions the exchangeable cations can dissociate from the particle surfaces to form diffuse double layers. This leaves the surface negatively charged and sets up a potential difference between the surface and the bulk solution, the concentration of the cations decreasing with distance from the surface. The effective thickness of the diffuse double layer is smaller the greater the concentration of the salt solution, and a quantitative study by Gouy (1910) was revived and greatly extended by Schofield (1948).

Ratio Law

When the salt solution contains ions of different kinds the distribution of cations away from the face depends upon their valencies: Schofield's ratio law (1947a) states the condition for equilibrium between the cations in the exchange complex and a smaller number in solution: equilibrium will be maintained if the concentrations of monovalent ions are changed in one ratio, those of divalent ions in the square of that ratio and those of trivalent ions in the cube of that ratio. As an example, if the concentration of a monovalent ion is halved the concentrations of divalent and trivalent ions must be decreased to one-quarter, and one-eighth of their former values. (One practical aspect of this is the possible effects of percolating rain or irrigation water on the nutrient balance of the soil, and on its permeability—see below.) For a calcium clay in equilibrium with a solution of calcium chloride the ratio of the square root of the calcium concentration to that of hydrogen ion should not alter. To test the law over a wide range of concentrations it is better to use "activities"—*a*—rather than concentrations, and as a ratio is not altered by multiplying both terms by the same quantity, the equilibrium condition can be written

$$\mathrm{pH} - \tfrac{1}{2}p\,\mathrm{Ca} = p(\mathrm{HCl}) - \tfrac{1}{2}p(\mathrm{CaCl_2}) = \tfrac{1}{2}\log a_{\mathrm{Ca(OH)_2}} + 14{\cdot}2$$

where p denotes the negative of a common logarithm and 14·2 is the negative logarithm of the ionisation product of water. This is the form of the relationship expressed in activities of neutral substances and gives an estimate of the activity of $Ca(OH)_2$ in the system. Schofield and Taylor (1955a) confirmed the accuracy of the equation for calcium clays over a wide range of concentrations of $CaCl_2$, and made similar measurements on aluminium, potassium and sodium clays. They (1955b) also extended the general reasoning to normal non-saline soils where the chief exchangeable cations are calcium and magnesium, recognising that pH as ordinarily determined will depend on the activities of other ions in the system. They found that constant values of the quantity $\text{pH} - \frac{1}{2}p(\text{Ca} + \text{Mg})$—the so-called "lime potential"—were obtained in solutions of calcium chloride up to 3×10^{-2} M. From this they suggested that for routine measurements of soil pH, $M/100$-$CaCl_2$ solution should be used instead of water, and a soil/solution ratio of 1/2 was recommended, though the exact value is not critical. The suggestion and the recommendation are widely used.

Negative adsorption and swelling pressure. As cations are attracted to the negatively charged surface, so anions are repelled—so-called "negative adsorption"—and the increased concentration of a solution in contact with clay can be used to measure the surface area of the clay (Schofield 1947b). When the clay particles are so close together that their diffuse double layers interact, new aspects of charge distribution become important. For two fixed parallel clay plates the combination of attracted cations and repelled anions produces a total ionic concentration at the mid-plane greater than that in the bulk solution. For equilibrium of the water throughout the system some water must attempt to move into the space between the parallel plates to compensate for the lowering of the free energy by the additional ions there: squeezing more water in increases the hydrostatic pressure, and unless there is some cohesive force to counteract the pressure (one of the attributes of a soil with stable structure), the clay will swell and ultimately deflocculate. Alternatively, suction can be applied to the bulk solution. From measurements on sodium montmorillonite, Warkentin and Schofield (1960, 1962) concluded that the osmotic pressure of the ions in the diffuse layer accounts satisfactorily for swelling pressures in dilute salt solutions ($<10^{-3}N$-NaCl): for more concentrated solutions the observed pressures were relatively higher than those calculated, and this was explained by a higher activity of the ions in the diffuse double layer, as compared with the neutral equilibrium solution. Assuming the repulsive force was correctly estimated, there was no evidence for attractive forces of the order of magnitude of the osmotic repulsive force. However, the measured X-ray spacings for sodium montmorillonite in salt solution, e.g., Norrish (1954), are usually considered as representing an equilibrium between the repulsive pressure and attractive forces, and it seems there are still some difficulties to be resolved.

In most agricultural soils the predominant cations are calcium and magnesium. When they are partly or wholly replaced by sodium, as by sea-water flooding, or by the use of unsuitable irrigation water, there may be unfortunate effects on the stability of the soils. The structure is stable provided the electrolyte concentration is great enough, but loss of permeability, caused by swelling and deflocculation, occurs when attempts are made to wash out the salt, unless the sodium is replaced by calcium in the exchange complex. Quirk and Schofield (1955) measured the changes in permeability of soils saturated with one ion only, using solutions of the chloride of that ion, and made similar measurements on sodium–calcium clays with differing degrees of sodium saturation, using solutions of mixed chlorides diluted according to the ratio law. The results showed the importance of knowing the values of $[\mathrm{Na}]/[\mathrm{Ca} + \mathrm{Mg}]^{\frac{1}{2}}$ for the soil and water to decide whether the water was safe to use. For sites already damaged, Schofield and Taylor (1961) devised a quick method of estimating how much gypsum must be added to soil affected by sea-water to prevent structure being damaged by the leaching action of rain water. This was used by Advisory Officers after the North Sea floods of 1953.

Soil Structure

Seedbed preparation, and other aspects of soil management, are designed to produce a good "crumb" structure—open agglomerations of sand, silt and clay particles held together in some way, or ways. In a soil with good natural structure the crumbs are resistant to slaking, i.e., dry crumbs do not disrupt on wetting and, when wet, they remain strong enough to resist destruction by mechanical disturbance. The necessary cohesion to withstand swelling pressure can come from organic matter, and knowledge of the electrical properties of the clays provided useful guidance in the design and interpretation of experiments on soil structure (Emerson, 1957). Emerson and his colleagues sought for the type of bonding possible in natural soils by studying the action of synthetic polymers, ionic and non-ionic, in modifying the swelling behaviour of sodium-saturated crumbs (in effect, using the swelling pressure as a calibrated hammer to try to disrupt the crumbs). The results showed that polyvinyl alcohol was taken up by the planar faces by hydrogen bonding, whereas carboxylated compounds formed complexes on the edge faces of clay crystals. Crumbs from pasture land are stronger than those from arable land, but differ little in rate of uptake of water, degree of swelling or in appearance in thin sections (Emerson and Dettmann, 1959). Emerson (1959) therefore concluded that the clay crystals in crumbs form domains of oriented crystals free from organic matter, but these domains are linked to the quartz particles by organic matter, mainly at the planar faces, but carboxylated compounds could also link with the edge faces of the domains. The domains themselves could be linked to each other, to give an open structure amid the mineral particles but still able to restrain diffuse double layer swelling of the clay when sodium-saturated. Flocculation by edge-to-face attraction between clay units is considered unlikely.

Measurement of Electric Charge

The possibility of such edge-to-face attraction depends on the fact that in some conditions the edges of the clay crystals do carry a positive charge:

the presence of the negative charge on the planar faces would then provide an electrostatic mechanism for flocculation and structure building. Schofield (1949), working with a clay subsoil, found that the total charge varied with the pH of the clay suspension. An increase in the negative charge with alkalinity was explained as caused by dissociation of hydrogen from hydroxyl groups attached to silicon. In an acid medium (pH 2·5) chloride ions were attracted to the clay, showing that it carried a positive charge, but after chemical treatment that removed free oxides chloride ions were repelled at pH 2·5. Schofield concluded that the positive charges were carried by the free oxides. Later, Schofield and Samson (1953, 1954) found that sodium kaolins were firmly flocculated in distilled water and absorbed chloride from neutral sodium chloride solutions; they explained their results by the formation of positive charges on the edges of the clay, produced by proton transfer to oxygen atoms or hydroxyl groups on the exposed edges. Adding alkali caused deflocculation, and this was explained by neutralisation of the positive edge charge and the loss of edge-to-face attraction. The implications for anion adsorption and other aspects of the electro-chemistry of the surface were discussed by Fieldes and Schofield (1960). As subsequent work has produced a very different explanation, a brief note on technique is necessary. The kaolin was prepared by first washing with *N*-NaC1 adjusted to pH 3 with HC1 in the belief that the dilute acid would remove all aluminium external to the lattice, without causing any significant breakdown: the kaolin was then washed with distilled water until free from chloride. The assumption was that the system was electrically neutral, the zero charge balance being made up of permanent negative charge on the planar faces incompletely balanced by sodium ions—and only sodium ions—with permanent positive charges on the edges to account for the remainder. There is now little doubt (Cashen, 1961) that the assumption was wrong: kaolins prepared in this way contain considerable quantities of exchangeable aluminium, produced by acid attack, and this can account for the observed flocculation, and for other properties of the treated clay, without invoking any permanent edge charge.

Reactions of clays with acids. Acid treatment will produce a hydrogen clay, but in agricultural research it has long been known that such clays, however prepared, are unstable, and, in time, change to the aluminium form. The process is probably controlled by diffusion of hydrogen and aluminium ions to and from the edges of the crystals, and will be irreversible. As first proposed by Paver and Marshall (1934), it is reasonable to suppose that the presence of a positive charge on an edge represents an intermediate stage in the release of aluminium ion from the lattice; also that protons transferred from the surrounding electrolyte will act more quickly than hydrogen ions that have to diffuse from the planar faces. For *N*-NaC1 at pH 3 the ratio of sodium to hydrogen ions is 1,000 and there should be very few exchangeable hydrogen ions, yet a positive charge is formed in such a strong salt solution. It is possible that the assumed "permanent" model of Schofield and Samson is, in fact, a good description of a transient state, after washing with distilled water, in which the incompletely sodium-saturated clay has equal amounts of unbalanced negative and positive charge (faces and edges). The electrical stress between the faces and edges might produce physical breakdown at the edges releasing aluminium to replace the missing cations on the planar faces and so discharging the edge faces (Cashen 1959). The detritus would contain other lattice components, notably potassium, and similar processes in the soil, acting very much more slowly, of course, might account for some release of non-exchangeable potassium from mica-type minerals in the soil, as measured by P. W. Arnold and B. M. Close (*Rothamsted Report* for 1961, p. 57). Further, an initially neutral soil that had become acid because of decomposing organic matter could not have its acidity washed out if there are exchangeable aluminium ions present: these can supply hydrogen ions on hydrolysis.

Hydrolysis of aluminium ion. In addition to its bearing on the permanence and importance of edge charges, the behaviour of aluminium in the soil is clearly the key to problems of soil acidity. As part of recent work on clays (Cashen, in preparation), experiments with dilute solutions of alum ($2{\cdot}78 \times 10^{-4}$ *M* in Al) have followed the changes in electrical conductivity as solutions are titrated with dilute alkali. There is some evidence —perhaps significant—of the formation of an ion $AlOH^{2+}$ as an intermediate stage in the formation of a complex ion $Al_6(OH)_{15}^{3+}$ (or one with the same OH/Al ratio) already suggested by Brosset, Biedermann and Sillén (1954) as the main product of the hydrolysis of aluminium in the acid region. The reactions are

$$6Al^{3+} + 6H_2O \longrightarrow 6Al(OH)^{2+} + 6H^+$$
$$6Al(OH)^{2+} + 9H_2O \longrightarrow Al_6(OH)_{15}^{3+} + 9H^+$$

At this stage the aluminium is five-sixths neutralised. Electro-chemically, the new complex ion is equivalent to one aluminium ion; physically—in the clay—it produces some striking effects.

Continued titration takes the system through the neutral point without very clear evidence of where it is, and the next clear singular point is assumed to correspond to the formation of aluminate, according to

$$Al_6(OH)_{15}^{3+} + 3OH^- \longrightarrow 6Al(OH)_3$$
$$6Al(OH)_3 + 6OH^- \longrightarrow 6Al(OH)_4^-$$

The important quantities of alkali additions are: to aluminate, total 24 OH^-; to hydroxide, 18 OH^-; to complex ion, 15 OH^-; giving ratios of 4/3:1:5/6, or 8/5:6/5:1.

The same three phases were detected in similar experiments with the ions displaced from an aluminium kaolin by a solution of cetyl trimethyl ammonium bromide. After necessary corrections the ratio of hydroxyl additions for the aluminate stage and the complex ion was between 1·57 and 1·62, close enough to 8/5 to show that the ions displaced from the kaolin, prepared by acid washing, were Al^{3+} and not others hydrolised during the preparation of the clay.

In a direct titration of the aluminium kaolin the three phases were not so clearly differentiated, but the ratio of additions for aluminate and hydroxide was near 1·33. The very close agreement with the ratio 4/3 is probably fortuitous, but this is the ratio to be expected if the edge charges are playing no significant part in the neutralisation. It is concluded that the effects of the edge charge are small compared with those of the permanent charge on the planar faces.

Physical Properties of Suspensions

Schofield and Samson (1954) found that a "sodium" kaolin was flocculated in electrolyte-free conditions, and explained a completely unexpected result by invoking edge-to-face bonding dependent on a permanent positive edge charge. It now seems clear that the clay was a sodium/aluminium clay, that the flocculation is attributable to the aluminium and that behaviour after adding alkali can be accounted for by the hydrolysis of the aluminium. One of these effects is the extremely large decrease in viscosity of the suspension, which occurs after the five-sixths stage of a neutralisation of the aluminium. With their "sodium" kaolin the decrease was to a thousandth when 1·0 milli-equivalents of alkali had been added (per 100 g clay); with the pure aluminium clay the amount needed was 2·5 milli-equivalents. It is a fair inference that the sodium clay was 40% saturated with aluminium ion, and all the electro-chemistry and rheology can be explained on this basis.

Buzágh and Szántó (1960) found that "hydrogen" montmorillonite exhibited thixotropic behaviour when 80–90% neutralised. As bonding between particles is usually a requisite for gel formation, Cashen (1963) suggested that the clay was an aluminium clay, and that the bonds were formed by shared complex ions. The maximum opportunity for such bonding should occur at the 5/6 stage of neutralisation (83%) when all the aluminium initially present is still there, retained as complex ions balancing one-sixth of the charge: the remaining five-sixths of the permanent negative charge is balanced by sodium ions, capable of exerting diffuse double-layer repulsion in the ordinary way. The distribution of sodium and aluminium at this five-sixths stage may be random, but because the aluminium ions have to move to form the complex ions, the same mobility may permit them to take up positions corresponding to minimum energy, i.e., as far apart as possible under their mutual repulsions. This might produce a tendency to move towards the periphery of the surfaces, producing a polarisation of the particles.

Whatever the explanation, the effect is real. Simple experiments on aluminium kaolin and aluminium montmorillonite show that the viscosity is maximum near 83% neutralisation. Elsewhere, Nash (1960) reports a maximum near 80% for aluminium montmorillonite. Nor need the effect be peculiar to aluminium and sodium: Cashen (1963), in discussion, suggests that it might apply to other mixed ionic systems with the ions greatly different, so explaining the rheological behaviour of materials free from clay or aluminium.

As indicated previously, the effect of swelling pressure can be balanced by applying suction to the external liquid. New measurements of this equilibrium suction (Cashen, in preparation) on five-sixths neutralised aluminium clays show that the responses of suspensions of montmorillonite and kaolin to shearing stress are not always the same.

Conclusion

All of this work grew out of the pioneer activity of Schofield, and it is appropriate to return to a sentence from his 1947 review. "The undertaking is formidable, and much groundwork is needed before results can be obtained that bear directly on the very complex conditions existing in field soil." Under nearly every heading in the present review there is evidence, or a hint, of progress towards Schofield's goal. The ideas behind the ratio law are relevant to many aspects of the percolation of rain or irrigation water, both in chemical effects (leaching and cation exchange) and in physical effects (swelling and decrease in permeability): our knowledge of how soil structure is produced and maintained is better than it was, with understanding of the role of organic matter; there is a clue to one aspect of weathering that may release the stored potash reserves of the soil; in the study of this the problem of soil acidity is clearly revealed as the problem of aluminium in the soil, and in measuring acidity for research and advisory work it is worth while using the régime of stable lime potential. The newest work, here only mentioned in advance of publication, brings the department back to topics that were major projects before 1937—the flow properties of clays. Fresh thinking about these with new ideas may help in problems of soil management both in agriculture (e.g., cultivation operations) and in civil engineering (e.g., foundation problems).

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APHID NUTRITION AND REPRODUCTION

C. J. BANKS

Aphids owe much of their success as plant parasites to a special method of feeding on plant sap, combined with an extraordinary method of reproduction that enables them to multiply and exploit their host plants rapidly (Kennedy and Stroyan, 1959). They have also evolved an efficient method of dispersal that enables them to move rapidly from one temporary host to another. Dispersal has been intensively studied at Rothamsted, but less attention has been paid to the food requirements of aphids, and their nutritional needs are very imperfectly understood. However, a proper understanding of such problems as host-plant selection, the basis of resistance by plant varieties to infestation and perhaps the transmission of plant viruses by aphids, may well depend on better information about aphid nutrition.

Aphids multiply faster on some host species than on others; for example, *Myzus persicae* increases faster on *Brassica* spp. than on sugar beet, spinach or lettuce (Heathcote, 1962), and *Aphis fabae* faster on beans than on sugar beet (Banks and Macaulay, unpublished results). They also grow faster and reproduce more on some varieties of plant than others, for example of pea (Auclair, 1959), wheat and barley (Painter and Pathak, 1962), raspberry (Hill, 1957) and beans (Davidson, 1922; Müller, 1951; Tambs-Lyche and Kennedy, 1958). They reproduce faster on young parts of plants than on older parts (Kennedy and Booth, 1951; Banks, 1958; Heathcote, 1962) and more on senescing parts than on mature parts (Kennedy and Booth, 1951). These different rates of increase might indicate differences in the nutritive quality of the sap, but might depend on different rates of feeding or assimilation.

Before the amount of food ingested could be related to the aphid's growth, reproductive rate and fecundity, a satisfactory method of studying the insect's feeding rate throughout its lifetime had to be developed. Much of this article deals with recent developments at Rothamsted on the feeding and reproduction of *Aphis fabae*, the bean and sugar-beet aphid, about whose anatomy and biology so much is already known (Davidson, 1925; Weber, 1928).

Method of feeding. Aphids were thought to suck plant sap in the way the Heteroptera or bugs do, until Kennedy and Mittler (1953) showed that when the stylets of *Aphis fabae* (feeding on bean plants) and of *Tuberolachnus salignus* (feeding on willow stems) were severed, sap continued to exude from the stylet stumps still embedded in the plants. Mittler (1957) found that sap exuded from the stumps at about the same rate as intact *T. salignus* excreted honeydew while feeding on the same willow plants, and that it sometimes continued to exude for days. Kennedy and Mittler suggested that the pressure of sap within the phloem sieve tubes, where these aphids usually feed, was responsible for forcing the sap into the feeding aphid. This advance in knowledge, confirmed by von Dehn (1961) with several other aphid species feeding on herbaceous plants, led to the assumption that the aphid feeds continuously and passively once its stylets have tapped a sieve tube element, and that the sap is forced through the gut of the insect by the pressure within the plant.

Banks and Nixon (1958), following a suggestion of Herzig (1937), advanced the subject further by showing that *Aphis fabae* feeding on fully turgid bean plants excreted and fed faster when attended by the ant *Lasius niger* and that excretion slowed to its initial rate when the ants were removed. They suggested that the so-called "sucking pump" in the aphid's head probably acts as a valve that opens periodically and allows some sap under pressure to enter the insect's gut; the relaxation of the muscles of the pump attached to the head wall would close it, and their contraction would open it again when another meal of sap was to be ingested. When attended by an ant the aphid could open the valve more often and thus increase the rate of feeding. To feed continuously, however, the muscles opening the valve would have to remain continuously contracted, something no muscle does. Whatever the mechanism whereby the aphid controls its rate of feeding and excretion, this work showed that the aphid does not feed continuously, and does not depend solely on pressures within the plant while feeding on a turgid plant.

That some aphids can suck when they have to, is shown also by work of Mittler and Dadd (1962) and of Auclair and Cartier (1963), who reared *Myzus persicae* and *Acyrthosiphon pisum* that fed through stretched Parafilm membranes on liquid diets under negligible pressure. Nevertheless, many if not most aphids probably use the pressure of the sap for normal feeding, and only under unusual conditions, perhaps when the plant wilts, are they forced to suck. Some aphids feeding in galls and pseudo galls may feed by sucking, but little is known about their feeding behaviour.

It is generally thought that an aphid's stylets usually penetrate a sieve tube element in the phloem, where they reach sap under continuous pressure; so the aphid feeds with a minimum of effort and at the same time obtains a food rich with the plant's own nutrients. There is abundant evidence that aphid stylets penetrate to the region of the phloem but little to show that they habitually end in sieve elements. Phloem sap, still under pressure, may pass from sieve elements into nearby parenchyma cells where the stylet tips have penetrated, but the movement of phloem sap is imperfectly understood.

Feeding and excretory behaviour. The feeding and excretory behaviour differs in different species. *Myzus persicae* is said to be a restless aphid that often changes its feeding site, whereas *Aphis fabae* stays and feeds at the same spot for days, and when disturbed often has great difficulty in withdrawing its stylets. Other species, for example, *Acyrthosiphon pisum*, *Megoura viciae* and *Microlophium evansii* to mention a few, immediately withdraw their stylets and fall off the plants when disturbed. Such a different response suggests that these aphids might differ in their feeding habits.

Broadbent (1951) described the different ways in which aphids excrete. Larvae of 'free-living' forms (those that feed on the leaves and stems of plants and not in galls or pseudogalls) kick the honeydew droplet from the anus with a hind leg; the adults flick the droplet off with the cauda (tail). But the behaviour of aphids of this kind also differs; for example, *Aphis fabae* adults flick the droplet vertically downwards (Banks, 1958), whereas adult *Megoura viciae* flick the droplet downwards but to one side. The details of feeding and excretory behaviour of various aphid species deserve further study. The adult apterous virginopara is usually regarded as a neotinic, that is, a morph that retains many larval characters in the adult stage; the "metamorphosis" of the larval alata at its final moult is suppressed in the aptera, and morphological changes at the final moult are therefore extremely slight (Lees, 1961, p. 75). However, the conclusion seems to overlook the fact that the acquisition of a large cauda by the adult aptera and alata is a very prominent morphological change, which is also accompanied by a dramatic change in excretory behaviour.

Measurement of the feeding rate of *Aphis fabae*. Feeding rates of aphids have been measured hitherto usually for short periods and by various methods, some of them unsatisfactory. Watson and Nixon (1953), Day and Irzykiewicz (1953) and Banks and Nixon (1959), for example, estimated the feeding rates of aphids for periods of a few hours by measuring the radioactivity of insects that had fed on radioactive plants, but the measurement may not be valid because the concentration of the radioisotope of the sap actually ingested was unknown. The rates of feeding of various aphids obtained by different workers differ very greatly (Auclair, 1963), and many results imply that the rate rises to a maximum and then stays constant.

Before any attempt could be made to link the rate of ingestion with reproductive capacity it was essential to have a method of recording the feeding rate continuously over long periods, if possible for the whole of the insect's lifetime, and at the same time record its reproductive rate and fecundity. The only satisfactory method is that of Auclair (1959) who estimated the sap ingested by the larva of *Acyrthosiphon pisum* from pea plants by adding up the increase in body weight, the loss of water by evaporation, the weight of the cast skins and the weight of the honeydew excreted during 71 hours. Banks and Macaulay (1964) extended this method to estimate the sap ingested during the whole lifetime of *Aphis fabae* from two varieties of field bean, and recorded the growth, reproductive rate and fecundity of the same insects. They used the fact that most of the sap ingested is excreted and that the rate of excretion is a good index of the feeding rate so long as the environment is reasonably constant (Mittler, 1957). Excretion is affected, among other factors, by changes in temperature, air humidity, wind and the turgor pressure of the plant (Mittler, 1962), so Banks and Macaulay made their experiments under as constant conditions as they could maintain, and they used aphids of one kind only: the apterous progeny of alate virginoparae produced from a clone.

Temperature and lighting were kept constant; air was kept humid to lessen evaporation from the insects, and air movements were eliminated.

As the honeydew clocks used by other workers are bulky and expensive, a compact, inexpensive machine was made to collect simultaneously all the honeydew excreted by ten individual aphids feeding each on its bean plant, to record their daily total excreta continuously from birth to death. Two steel rods rotating slowly and continuously wound up ten long strips of chromatography paper stained with an indicator to reveal the honeydew droplets that fell on them as they passed under the aphids. The papers, which were long enough to last for 24 hours, were replaced each day; thus, all the honeydew droplets from each of the ten aphids was collected and counted throughout the insects' lives, and an estimate of variability obtained.

Honeydew was also collected daily in mineral oil from other aphids of the same age on similar plants to measure the size of the droplets. The average excretion rate was estimated from the product of the mean frequency of excretion and the mean size of the droplets, and this volume of liquid, when multiplied by the specific gravity of honeydew, separately determined, gave the total weight of the excreta. The sum of the weight of the honeydew, water lost by evaporation, the increase in body weight of the insects and the total weight of their larvae born estimated the total weight of the sap ingested. The reproductive rate, fecundity and the length of the reproductive and post-reproductive life of the same aphids were also recorded.

The larval aphid ingested 3·5–4·5 mg sap in a life of 7 days and the adult about 30 mg sap during a reproductive life of 21 days; only 10% of the ingested sap was used for growth and reproduction and 90% was excreted. The excretion rate of the adult increased to a maximum of 30–40 drops per day on the third day of adult life and then started to decrease. This decrease was apparently because of changes within the insect itself, for although the aphids were transferred to fresh plants every 5 days, the excretion continued to decrease.

The rate of feeding therefore also declined as the aphid aged. We can but speculate as to the cause; muscle degeneration or a gradual blocking of the stylet canal or gut (see also below) or, less likely, a progressively more efficient use of sap with increasing age, may be explanations; but whatever the reason, it is important to recognise that the rate *Aphis fabae* feeds is far from constant and that it rises quickly to a maximum and then declines with age. Other species probably have their own patterns of excretion and feeding, and comparisons of the feeding and excretion rates of different species or of the same species on various host plants should be based on complete records throughout the insects' lives. The large variations in rates of feeding listed by Auclair (1963) can probably be explained partly by differences in age of aphids and in their patterns of feeding; feeding rates will also probably be affected by differences in size of insect and the kind of host plant. So far as I am aware, feeding patterns of other aphid species have not yet been established, but if other aphids behave as *Aphis fabae* does, the loss of ability of older aphids to transmit plant viruses, reported for some species, may be associated with a decline in the feeding rate and what seems to be a change in the physiological state of the insect as it ages (Bawden, 1964, p. 130).

Reproduction. Adult insects usually need to ingest food to reproduce. The female in particular needs food to make materials to form yolk. In some species the reserves are built up during the larval stage, especially in those species whose adult insects have only vestigial mouth parts. In others, the reserves accumulated during larval life are supplemented by a carbohydrate diet during adult life, and some adult insects need a special protein meal before they can reproduce (Wigglesworth, 1960).

Aphids are, however, unusual insects because they reproduce parthenogenetically and viviparously over many generations, and some species reproduce in no other way. In the parthenogenetic aphid, therefore, the relationship between reproduction and food ingested is more complicated than in oviparous species of insects. The parthenogenetic adult contains embryos in all stages of development from eggs to mature larvae, and the more mature of these also contain embryos in an early stage of development (see below); an aphid larva is in effect starting to reproduce before it is born. Thus, an adult parthenogenetic aphid nourishes not only her daughter embryos but also some of her granddaughter embryos (Lees, 1959; Kennedy and Stroyan, 1959). It is the telescoping of the generations in one individual, as Kennedy and Stroyan have aptly put it, combined with the specialised method of feeding, that enables aphids rapidly to multiply and exploit their host plants.

During the first 2 weeks of reproductive life, adult *Aphis fabae* gave birth to 5 or 6 larvae daily, but only about 2 during the following week. Reproduction then ceased fairly abruptly, although excretion, which was also decreasing, continued intermittently for a few more days. The decline in the reproductive rate as the insect ages is more apparent when the number of larvae born in successive 5-day periods is considered rather than the numbers born daily (Banks and Macaulay, 1964). It then appears that the numbers of larvae born increases to a maximum of about 30 in the first 5–10 days of adult life and thereafter decreases. The maximum of the reproductive rate lags behind the maximum of the feeding rate, which occurs on the third day of adult life.

Reproductive life was followed by an almost equally long post-reproductive period. During this time the aphids remained with their stylets inserted into the leaves, the abdomen swelled and the insects increased in live weight and especially in dry weight (Banks and Macaulay, 1965), showing that, although excretion had stopped, small quantities of sap were still ingested. The comparatively large increase in dry weight in early post-reproductive life was not accompanied by a pronounced increase in fat content (Banks and Macaulay, unpublished results), although many oil globules occur in the body cavity once occupied by the tightly packed embryos. Sugars may accumulate during this period, probably because the aphid continues to feed but not to excrete. *Myzus persicae* and *Aphis fabae* have much larger crops when they feed on plants of the Chenopodiaceae (which includes sugar beet) (Moericke, 1960) than when feeding on other plants. Edwards (1965) examined the gut of *Aphis fabae* feeding on chenopods and discovered solid, insoluble polysaccharides in the crop, formed apparently as a secretion from the crop epithelium. It seems that aphids, feeding on plants containing an excess of sugar in the sap, and being unable to excrete faster to dispose of it, convert soluble sucrose from the sap into insoluble forms that are retained in the crop. Such a process perhaps also occurs in aged *Aphis fabae* that continue to feed on beans but not to excrete, and an accumulation of sugars might account for the increase in dryweight. Unlike *Aphis fabae*, the willow aphid, *Tuberolachnus salignus*, excretes large quantities of honeydew during post-reproductive life (Mittler, 1958), but this lachnid possesses a so-called filter chamber, one of whose functions could be to transfer unwanted excess sugar rapidly from the fore gut to the hind gut. *Aphis fabae*, like most other aphids, has no filter chamber, and excretion in old age may stop if the gut is gradually blocked with solids.

Banks and Macaulay (1964) found that the fecundity (total larvae born) of apterous *Aphis fabae* was remarkably constant, no doubt because they used a clone of aphids in a constant environment. Because reproducible results were obtained in this way, fecundity and reproductive rate are considered to be better criteria of the aphid's performance on a particular host plant than the multiplication rate of groups of aphids. On one batch of field beans (called variety A) the aphids consistently produced 89 ± 1·3 larvae and only 85 ± 1·3 larvae on another batch (variety B) in a reproductive life of 3 weeks. The difference, although small, is statistically significant. Taylor (1959) showed that on a variety of broad bean apterous adults of *Aphis fabae* produced an average of 96 ± 2·0 larvae during their lifetime, although his experimental conditions differed somewhat from those of Banks and Macaulay. Yet from the varieties A and B the aphids ingested the same amounts of sap; the difference in fecundity was not caused by the ingestion of different amounts of sap but probably by a difference in nutritiousness of the sap of the varieties.

Influence on fecundity of quantity and quality of nutrients ingested. The main component of the solid matter of the sap of plants is sugar (sucrose), much of which the aphid cannot use; nitrogen, dissolved in the sap as free amino acids and amides (Mittler, 1958; Zimmermann, 1960), is especially important for the synthesis of the protein essential for the aphid to grow and reproduce. Results of Auclair, Maltais and Cartier (1957) suggested that varieties of pea resistant to *Acyrthosiphon pisum* contained less free and total amino acids than susceptible varieties. The nitrogen in the plant sap could thus be a limiting factor in aphid development.

The differences in fecundity of *Aphis fabae* feeding on the field bean varieties A and B could have depended more on differences in the quantity and quality of the nutrients dissolved in the sap than on the total quantity of sap ingested. Differences in quality of sap rather than in rates of feeding were suggested earlier as the cause of differences in reproductive rate. For example, although larvae of *Aphis fabae* excreted and fed faster when attended by the ant *Lasius niger* (Banks and Nixon, 1958), as adults they did not reproduce faster than ant-free aphids (Banks, 1958). The greater multiplication rate of ant-attended aphids was attributed to the fact that they stayed longer on the young parts of the bean plants, where they reproduced faster, than on the old parts, where they reproduced more slowly. The reproduction of the ant-attended aphids was increased, it was suggested, because they benefited from a more nutritious sap on young parts of plants rather than from an increased flow of sap from which they could not assimilate nutrients faster.

The amounts of total nutrients ingested and assimilated by *Aphis fabae* from the two bean varieties was therefore measured throughout the whole life of the insect, to see if they were used in different ways (Banks and Macaulay, 1965). The indirect method of Auclair and Maltais (1961), who estimated the total nitrogen taken by *Acyrthosiphon pisum* larvae from varieties of pea during 72 hours, was extended to estimate the total solids (total nutrients) and total nitrogen taken by *Aphis fabae* from bean plants of varieties A and B throughout the whole larval and adult reproductive life. The total solids ingested approximately equal the increase in dry weight of the larva during development plus the weight of its four exuviae and of the solid matter of all the honeydew. Similarly, the total weight of solids ingested by the adult equal the sum of its increase in body dry weight, the dry matter of the total larvae it bears and the dry weight of the honeydew it excretes during its lifetime. Gases exchanged during respiration can be neglected. The total nitrogen ingested can be similarly determined by summing the nitrogen contents of these various components.

The larvae reared on variety B assimilated and excreted significantly more nitrogen than those on variety A, but this had no effect on reproduction for, as adults, they did not reproduce faster or produce more larvae, and those larvae did not contain more nitrogen than those born on variety A. The adults on variety A assimilated more total nitrogen because they were more fecund on it and they did not excrete more nitrogen in their honeydew. It seems that, although the aphids used the nutrients, particularly nitrogen, from the two varieties in somewhat different ways, this did not cause the difference in fecundity. The concentration of the nutrients, particularly nitrogen, in the sap of both varieties was apparently more than enough for reproduction, and did not determine the aphids' fecundity. The composition of the nutrients provided by variety A was possibly such that more larvae could be matured on it than on variety B.

The number of embryos in adult apterae. Because of the telescoping of the generations in the parthenogenetic aphid, nutrition during larval life affects the development of the embryos and the embryos within them to some extent. Good nutrition during larval life could allow more embryos to develop or to be matured faster, and thus could affect the early reproductive rate of the adult insect. But adult reproductive life is much longer than larval life, and the adult feeds more than the larva, so the nutrition of the adult would be expected to affect reproduction more than the nutrition of the larva.

According to Lees (1959), the newly born larva of the vetch aphid, *Megoura viciae*, has two differentiated embryos in each ovariole, and at the end of larval development there are six embryos in each of the 18 ovarioles, so that a newly moulted adult carries a potential of 108 larvae that it can bear during its lifetime; few further eggs are produced during adult life. Some embryos are resorbed and the usual number of larvae born is 97. In *Megoura viciae*, therefore, larval nutrition sustains the production of most of the eggs and the partial development of the embryos; adult feeding produces a few more eggs, but is mainly needed to complete the maturation of the embryos already differentiated.

By contrast, the newly moulted, unfed, apterous adult virginoparae of *Aphis fabae*, reared from alate virginoparae on field beans (variety B) under conditions outlined above, contain only an average of 36 embryos and egg cells, and such adult apterae produce an average of 85 larvae during their lifetime. Therefore, on the average, 49 more eggs and embryos (58% of the total) are developed and nourished to maturity entirely on nutrients ingested by the adult. We suppose, therefore, that the nutritiousness of the sap determines how many more than 36 embryos will be developed by the adult.

Linti (1960) counted the embryos and egg cells in apterous, alate and intermediate forms of *Aphis fabae* fed on mature detached leaves of *Phaseolus vulgaris* in damp petri dishes. Adult apterae contained an average of 54 egg cells and embryos, but many of the insects he dissected were not newly moulted and had fed and reproduced for an unspecified time, so could have had more egg cells and embryos than they contained when newly moulted; nevertheless, his adult apterous aphids contained many more embryos than Banks and Macaulay found in theirs. Linti did not say from what kind of parent (alata or aptera) his aphids came. The numbers of embryos differentiated and developed during larval life (depending on the state of larval nutrition) may depend, among other factors, on the parentage of the aphid; for example, adult apterae developed from alate virginoparae (like those of Banks and Macaulay) might contain fewer embryos initially than aphids born to apterous virginoparae; that is, the number of embryos may differ from generation to generation and even from morph to morph, and thus reproductive rate and fecundity could also vary. Aphid morphs differ mainly in structure but also differ in physiology (Lees, 1961), and the initial number of embryos contained in the newly moulted adult may be a characteristic of the morph, and thus an example of "physiological polymorphism" (Richards, 1961; Johnson, 1963). Larvae born to alate virginoparae are smaller and weigh less than those born to apterous parents (Banks and Macaulay, 1965) and might develop fewer embryos. The reproductive rates and fecundities of aphids of different generations are at present being examined further.

Effects of larval and adult nutrition on reproduction. The reproductive rates (numbers of larvae born per 5-day period) of the aphids reared on the field bean varieties A and B were nearly identical during the whole reproductive life. The small but significantly greater fecundity of the aphids on variety A was caused by a few more larvae being born *late* in reproductive life, which suggested that the adult aphids were somewhat better nourished on variety A and could mature more larvae than those feeding on variety B. However, more recent work suggests that the adult aphid's fecundity may also be affected primarily by larval nutrition. In any batch of larvae, all born to winged aphids on bean plants of variety B at the same time or within a few hours of each other, some develop and become adult sooner than others. The fecundities and reproductive rates of a batch of adult aphids that had developed quickly (fast developers) were compared with those of a late-developed group (slow developers). The fast developers produced significantly more larvae than slow developers, and the difference was double the difference between the fecundities of aphids reared on the two bean varieties. Further, differences in the reproductive rate were much greater during the first 5 days of adult life than during the next 5 days; thereafter the rates were identical. The difference in reproductive rate accounted for the difference in fecundity. The greater fecundity and reproductive rate of the fast developers was caused by events during *early* adult life and suggests a better nutrition during the larval life of the parent insects. Newly moulted fast and slow developers contained the same number of embryos, so fast developers must have matured their embryos more quickly during larval life and delivered them faster when they became adult. Fast developers were also heavier and larger than slow ones; they did not feed faster in the adult stage, but they must have assimilated food faster. When comparing the fecundities and reproductive rates of aphids it is important, therefore, to use aphids with similar rates of larval development. The causes of the greater fecundity of fast developers are not yet understood, but larval nutrition may affect fecundity by increasing the early reproductive rate of the adult. The difference in fecundity between aphids on the two bean varieties differs from that between fast and slow developers, and for another cause.

We have also recently discovered that the fecundity of aphids reared on variety B can be greatly increased by changing the conditions of growing plants. The mean fecundity of aphids on variety B was 85 ± 1·3 larvae, but it was 94 ± 1·5 larvae when aphids (born, as before, to alate virginoparae) were reared and kept under the standardised conditions described earlier, but on plants that had previously been grown in daylight with supplementary light from mercury fluorescent lamps. The aphids apparently received a still more nutritious sap from plants irradiated in this way, and had been under-nourished under the previous lighting régime.

There is, presumably, an upper limit to the number of larvae that an aphid can produce, and this maximum could be genetically determined and characteristic of the species or race. But reproductive rate and fecundity can depend on the conditions of rearing of aphids and plants, so it is essential to know the conditions that achieve maximum fecundity; also to adopt standardised conditions when testing host plants for 'resistance' and 'susceptibility' to aphid growth and reproduction, for a variety 'resistant' under one set of conditions may be 'susceptible' under others.

Thus, the apterous aphid's fecundity seems to depend primarily on its nutrition, that is, on the quality of the nutrients it gets from its host. The feeding rate and quantity of sap ingested are not basically important in deciding reproductive rate and fecundity of *Aphis fabae* on a suitable host plant such as the bean, although under some conditions or on other host plants the inability to feed at an optimal rate might well impair the assimilation of nutrients. The quantity of nutrients ingested from the two bean varieties did not determine differences in fecundity. It is the quality of the sap in the plant that seems primarily to affect the rate embryos develop during larval life, the rate of reproduction (especially during early adult life) and the numbers of larvae that the adult can mature; these features in turn affect the rate a colony of aphids will increase and the degree to which the aphid exploits its host plant. A great reproductive capacity is the aphid's main defence against natural enemies, and anything that decreases it makes the aphid more vulnerable to them.

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