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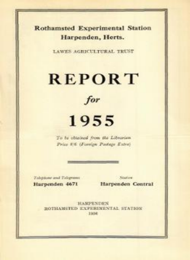

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ECOLOGICAL ASPECTS OF APHID FLIGHT AND DISPERSAL

By

C. G. Johnson

General Approach

There are two main aspects in the dispersal of insect populations: the process itself, and the significance of dispersal in the evolution and abundance of a species and in its status as a pest. We recognize, however, that both these aspects are part of the same ecological system and interdependent; and that the mechanics of dispersal can be fully appreciated only if placed in an ecological context.

This brief review considers the dispersal process itself in this way, as it concerns aphids: and deals particularly with some recent contributions, some of them unpublished, from Rothamsted.

In the past, especially in Britain, dispersal, and many other aspects of ecology, have often been studied by trapping flying insects and correlating numbers caught with weather factors. Usually this was done with a single trap a few feet above the ground, emptied once in 24 hours or even less frequently. This is very valuable for some predictions; but it is inadequate for an analysis of the biological and distributive processes in dispersal.

Trap data alone neglect the events in terrestrial ecology on which aerial abundance and so dispersal primarily depends. In fact, a trap abstracts the aerial situation from its ecological context, while a single trap at one level, emptied once a day, is an exceedingly blunt instrument for dissecting a process which extends hundreds or thousands of feet into the air and operates on an hourly rather than a daily time scale. This approach with aphids has kept alive for 20 years an erroneous hypothesis of dispersal which will be discussed later.

Nevertheless, trapping is necessary for measuring aerial density, if not always for analysing it; and a most necessary task was to improve the technique so as to relate numbers caught to the volume of air sampled and to record changes in density at hourly, or even shorter, intervals. Segregating suction traps have been developed to do this (C. G. Johnson & Taylor, 1955 *a* and *b*). Aphid dispersal, then, to be seen synoptically, must be studied both in relation to the insects on the crop and in the air as high as techniques will allow. This we have done, for the first time; and this article tries to put the whole study into focus.

It is helpful to consider the problems in three stages: the variations in numbers first liberated into the air from a primary source of supply and by subsequent flights; the dispersal of the insects through the atmosphere; and events leading to the return of populations to the earth and to alighting. Each stage has its collective, and also its individual, physiological aspects, which will be considered in the appropriate order.

APHID LIFE HISTORY

Readers unfamiliar with aphids may be helped by a general account of the life history: though the number of exceptions is bewilderingly great.

Dispersal is mainly by winged aphids (alatae) which often fill the air in millions. There are in general three main waves of dispersal during the year: in spring, summer and autumn; each builds up to a maximum and declines, and lasts from about 1 to 6 weeks. Though sexual forms occur (either male or female, or both, may be winged), most dispersal is by females, which reproduce without being fertilized and lay not eggs but small fully formed, immature aphids. These aphids cast their skins four times before becoming like the parent, parthenogenetic, viviparous adult females, which may be winged or not. Several generations a year may be produced. The proportion of winged (alate) to non-winged adults usually increases in dry weather and as plants get older. Sexual forms are often produced in the autumn, and the winter is often spent as eggs.

Some species attack a large variety of plant species, others are confined to one. Some species alternate regularly between different plant species in summer and autumn, the sexual generation being commonly found only in certain plants in the autumn.

VARIATIONS IN NUMBERS MIGRATING

A slight wind delays an aphid from taking off; and highest densities are commonly seen in calm weather. This has lead to the hypothesis that there is little dispersal above certain " optimum " weather conditions and that the amount of dispersal is controlled principally by weather acting on flight behaviour. Our results were at variance with this in nearly all respects (C. G. Johnson, 1954).

There are usually two elements in any change in numbers of insects flying; a changing population level and changes in the proportion of a population flying due to behaviour. In a human population the number of people on the streets from hour to hour is due to variation in behaviour not to population change; some insects are similar. But with aphids the population level of alatae changes so rapidly that it swamps behaviour in causing changes in numbers flying even from hour to hour. It is as if we produced a new family of immoderate proportions every time we went out for a bicycle ride and took them with us on subsequent occasions; and to understand what was happening we should have to investigate events at the source rather than by counting the people in the street. Similarly, if an insect trap is set up without care that it is near a primary source of alate production it catches many species of all sorts of ages indiscriminately, and the picture is blurred. But if we start at the beginning with a trap over a well-infested crop, producing large numbers of winged aphids, a clearer picture emerges (C. G. Johnson, 1952).

One of the characteristic features in the change of aerial numbers above such a place in the summer are the two peaks of relatively high density, one usually in the morning, another in the afternoon; and the virtual cessation of flight at night. It is not possible to explain this in terms of flight behaviour alone; two other factors operate. These are the changing rate of production of new alatae throughout the day and night and the permanent departure of these insects after the first take-off. This is the real cause of the periodicity.

The penultimate stage in alate production is the nymph: these moult with a marked periodicity into winged, parthenogenetic females which pass through an obligatory maturation period (from about 6 hours to 2 days) before flying away. For an analysis of density change above the crop these three stages, moulting periodicity, length of maturation period and the flight behaviour, had to be measured as they occurred consecutively in a natural population. This was done in 1952 with *Aphis fabae* in collaboration with L. R. Taylor and Dr. Else Haine.

Starting very early in the morning, the hourly rate of moulting was recorded on selected plants. On others all newly moulted alatae were marked, but with a different colour, every two hours: their departure time was noted and their maturation period found (the maturation period occupied almost the whole time between moulting and flight). The total number marked was about 3,000 during the 2½ weeks work. A suction trap gave the aerial picture; wind-speed and temperature were recorded every half-hour.

The maturation period is governed mainly by temperature, to which it is related in a typical sigmoid fashion. By using hour–degree developmental units it is now possible to estimate the length of the maturation period when the temperature is varying as in the field. Variation in this, together with the time of sunrise and sunset and the temperature threshold for take-off, can be used to reconstruct model curves of density change for any day which match with a satisfying accuracy those observed for the same day with a suction trap, whether they have one or more peaks. A full account of this process will be published shortly.

The basic pattern of flight rhythms is thus not caused by previous moulting rhythms as once suggested (C. G. Johnson, 1954). But there is a pronounced peak of moulting in the early morning, and there is no doubt that variable moulting rates modify the shape of the flight curve, and we are now able to assess this. The flight periodicity is therefore largely a population–developmental effect, weather-controlled flight behaviour playing a minor rôle except at the beginning and end of the day The causes of rhythms in moulting of nymphs into alatae are not fully understood: but they too may be due to a "piling up" of fully developed instars owing to variable rates of development. Haine (1956) has found that these rhythms persist even at constant temperatures; it is probable too that similar rhythms exist in other phases of aphid life—e.g., in parturition.

Summing up this aspect of the work, we can say that the basic causes of aerial density change from hour to hour and day to day (seasonal fluctuations are outside the scope of this review) over a source of alate production are now, in the main, understood for one species, *Aphis fabae*; and that, given only the hourly temperatures and times of sunrise and sunset, we can reconstruct the aerial density curves theoretically. Variations on the general pattern may be important, and other species need investigation to see if they agree with *A. fabae*. The first phase in the dispersal story is, to this extent, resolved.

But the analysis I have just described deals only with aphids flying away for the first time. It may be asked, do not all the subsequent take-offs of the gradually accumulating numbers of differently aged aphids swamp the limited number of first fliers? And does not the old hypothesis of weather-induced variation in take-off come back into its own?

There are several reasons why the old hypothesis cannot be re-established: with many species flight is limited to about 2–5 days by muscle autolysis (B. Johnson, 1953); the double peak often occurs well away from the source and suggests a limited degree of flight. Recent work by Haine (1955) has shown that while wind delays flight it does not prevent it, and several species of aphids can take off in considerable breezes (e.g., up to 7 m.p.h. for *A. fabae*); thus, in view of lulls and shelter in the field, wind is probably not a very important deterrent to flight except momentarily.

Moreover, because alatae production and flight is a continuous process during the day, and there are more windy occasions than calm ones, most dispersal actually occurs in winds from 5 to 20 m.p.h. (C. G. Johnson, 1954) not in the calm as once believed.

Müller & Unger (1954) interpret the double-peak periodicity of catches in yellow tray traps to variation in "alighting flight" (*Befallsflug*). This, we feel, is more likely to be reflection of the general density pattern rather than a variation in behaviour.

THE DISPERSAL OF APHIDS THROUGH THE ATMOSPHERE

It is well known that aphids reach great heights in the air; and we have shown that the majority flying are usually above about 100 feet. The aerial populations are frequently of astronomical proportions.

However, there seems to be a general idea that the aphids in the upper air are of no great significance in the general ecology of these insects. This is because it is assumed that either they die when carried aloft or come to nought elsewhere: it is thought that the important aphids are those few which manage to avoid these unfortunate excursions.

We do not take this view. Aphids do not die merely by being carried into the upper air: these aerial populations are alive. And though many of the individuals may never find host plants and survive to continue the race, nevertheless this kind of dispersal, and the behaviour which accompanies it (upward flight at take-off and subsequent flight in relation to host acceptability (see p. 198)), is of a kind to which the whole life and evolution of the species is attuned. There is no evidence which justifies the belief that it is a few which behave in some other way which are the only ones which need be considered.

We see the whole migrating population being physically mixed by turbulent diffusion high into the air and descending with variable frequency: and those finding the right place as belonging to this multitude. This section describes the events in the upper air from this point of view.

Aphids are weak fliers: they become dispersed vertically and laterally by very slight breezes. Where aphids are ubiquitous it is difficult, if not impossible at present, to follow their lateral displacement except over the sea or desert (Elton, 1925; Hardy & Milne, 1937; Wadley, 1931) or to obtain the decrease of density on distance from the source. However, many problems of aerial drift can be studied through the vertical dispersal. Other workers had shown that there is, in general, a decrease of density with height (Hardy & Milne, 1938; Glick, 1939; Freeman, 1945). But their attempts to correlate the number at any height with weather made little progress, for they did not distinguish the three elements determining it, namely population, activity and the distributive process caused by atmospheric circulation. Some features of the first two have been described in the previous section. We will now consider the distributive process irrespective of total numbers involved.

In order to measure the vertical gradient of aphid density and its changes it was necessary to trap at several heights simultaneously up to at least 1,000 feet. The trapping periods had to be short (about an hour at the most), consecutive and continuous during day and night through the whole season. Weather records at different heights were also necessary. This has been achieved with traps and meteorological instruments on a barrage-balloon cable at Cardington.

A convenient, though approximate, procedure with the data is to plot log aphid density (D) on log height (H). This gives a graph which can be regarded as linear for the present analysis. The gradient—that is the general distribution right up through the atmosphere—is then expressed as a single parameter, the regression coefficient b of log D on log H.

When the graph is steep and b is large, relatively more insects are in the lower atmosphere: this suggests less mixing than when b is small and there are relatively more aloft, though other factors, such as rates of supply and deposition, enter the picture.

By integrating this curve the total number of insects in any zone of the atmosphere or in the whole atmosphere can be estimated.

The change in gradient during the day

Flight starts early in the morning, and within the hour insects often reach great heights, where aerial populations usually continue to exist all day. By night-fall, however, as we have shown, there is a general decrease in total number of insects in the air (i.e., the integrated gradient) and the air is usually clear of aphids by daybreak. This has sometimes been taken to mean that the insects are precipitated at night, the same individuals having flown about all day; this, however, is not so. It is more rational and in accordance with observations to think of a continual replenishment from plants, a continual mixing up to hundreds or thousands of feet and a continual return of insects to the earth after a relatively short period aloft during the day; so that when mixing and replenishment die out towards evening the downward component dominates and the air clears.

If the atmosphere becomes stable, extremely rapid clearances of aphids from the upper air have been observed even during the day; so that within an hour or two higher altitude densities are too low to be measured. At the same time the fall of temperature with height (the lapse rate) becomes less. It is not known yet how far this is a general occurrence nor how the decrease of lapse rates are related quantitatively to the concentration of aphids at least into the lower air layers if not to earth. But it does suggest one explanation for the sudden, but quite common, appearance of swarms of aphids.

The double peak of density at crop level is often reflected in a double peak of total aerial numbers (the integrated gradient). This suggests that flight is then rather limited in duration to something less than the duration of a density peak—i.e., about 4 hours—and that on those occasions the first flight makes a major contribution to the daily numbers flying. But again the frequency of this pattern has yet to be measured before we can say how general it is.

However, though the aerial population in general descends by night-fall, it is common to observe extremely low densities high in the sky, and these may persist through the night and add up to a vast number of insects; and though the majority of the day fliers may sometimes travel no farther than a wind of 2 or 3 hours' duration will carry them, the low residual densities appear to indicate that a few persistent fliers may travel much farther. This also is now being put on a quantitative basis and in terms of frequency of occurrence.

We think thus of a continuous upward and downward as well as lateral movement, caused by mixing air masses varying in speed and intensity, and causing continual elevation and descent (sometimes to the earth) of variable numbers of aphids during the daytime; and that it is on some of these aphids, not on a few which escape this process, that the future of the race depends. Low-altitude concentrations or the chances of repeated ascents depend not only on the flight behaviour of the aphids but on atmospheric stability.

Variation in the gradient during the season

1948 was an exceptionally good season; the gradient was measured in 2-hourly intervals during the day and showed a strong seasonal trend. Low values occurred in spring and summer, suggesting maximal upward transport, with about 80 per cent of the aphids commonly above 100 feet; very high values of b with massive low-level concentrations occurred in September and October—with about 80 per cent usually below 100 feet. Summer appeared to be a time of high-altitude dispersal, autumn of low altitude and perhaps more local spread. How is this associated with meteorology?

In general, temperature decreases with height, and the greater the difference the less stable is the air. In 1948 the summer, which was characterized by weak aphid gradients (much upward transport), also had high lapse rate (atmospheric instability) and the autumn, with its strong aphid gradients, by low lapse rates. Thus correlation of gradients with air stability during the day (see above) is paralleled by a similar situation during the season, though how general this is has yet to be discovered.

Expression of the gradient

It is possible to express the vertical gradient for aphids (and for many other insects too) very precisely with an empirical equation. Further, collaboration with Mr. J. G. Skellam of the Nature Conservancy, has put this on a rational basis conforming to a mathematical model of diffusion. This work is being related to that published previously (C. G. Johnson & Penman, 1951) and will, it is expected, enable gradient problems to be handled with greater ease and precision. It is hoped, too, that the parameters can eventually be resolved into meteorological and biological components.

SOME ASPECTS OF INDIVIDUAL FLIGHT BEHAVIOUR

The work on the vertical distribution of aphids has been described in general terms: a consideration of individual species of aphids is in progress. Moreover, it has been considered from the collective aspect, and this has its limitations—e.g., an aerial *population* can persist during the day, though the individuals which compose it may be constantly changing. A persistent aerial population does not imply persistent flight; for it it may be composed of different individuals, some taking off as others alight. It is necessary therefore to consider the flight of individual aphids, and especially the factors influencing its character and duration, and the ability of aphids to take-off repeatedly after the first alighting.

During the last three years much work has been done, especially by Bruce Johnson, on the relation between flight activity and host selection; and on the behaviour of aphids after flight. New and interesting lines of work have been opened, and it is impossible to do them justice in this space.

Aphis fabae taking off for the first time frequently flies more or less upwards, usually until out of sight; this behaviour is probably common with many species, though little appears to have been published on it. So strong an upward flight does not appear to be the rule after some subsequent take-offs, though this needs investigation. Although usually attributed to phototaxis, convection currents (which need be very gentle), the flight attitude and wing-beat character may at times influence upward flight, apart from the effects of light. Aphids thus, at least on the first flight and probably on subsequent ones too, usually reach regions where they are carried by the wind.

The mean and maximum duration of flight, and hence the frequency of different distances traversed, are unknown for any species. As suggested on p. 196, the majority of aphids in the air on many occasions are probably limited by air conditions or their own capacity to a flight of something much less than 4 hours, though a small proportion may exceed this. Duration and proportion will no doubt vary greatly, and will no doubt be affected by temperature, humidity and light intensity.

Tethered flight of new aphids on pins, of up to 8–12 hours with some species (C. G. Johnson, 1954), cannot safely be taken as indicating the duration of first flights in nature. For apart from the possibility of abnormal stimulation (B. Johnson, 1955) preliminary experiments with flying aphids on a counterpoise indicate that, though the wings may continue to vibrate, the amount of lift necessary to support the weight of the aphid in free flight diminishes, often within the hour. The problem of flight duration and distance in nature is extremely difficult to analyse; for individuals cannot be followed and the duration of populations in the air is not a guide to the duration of individual flight (p. 195).

Nevertheless, prolonged journeys of hundreds of miles out to sea have been recorded (p. 196), which indicate that some individuals remain, if not in full and continuous flight, airborne for many hours. The 800 miles recorded by Elton (1927) for spruce aphids could, according to the numbers he saw, have been made by populations in very low densities comparable to those " residual densities " often seen at high altitude overnight at Cardington, a persistent whisp, so to speak, of the main migration.

Eventually the aphids return to the lower air (*Befallsflug* of Moericke (1955*a*) and Müller & Unger, 1952). It is possible, though not proved to be usual, that the aphids often seen flying about more or less horizontally, at crop level or slightly higher, in calm weather have recently experienced a fairly prolonged flight (pp. 195–6).

Eventually they settle on plants staying for periods from a few seconds to an hour or more or even permanently : during this time, even if it is short, they may probe, feed or even deposit young. Or they may do none of these things, but fly off again. This variation in behaviour in relation to flight is a major problem today.

Yellow tray traps 2 or 3 feet above ground level and away from a primary source of alate supply often show the double peak of numbers caught during the day. This has been interpreted by Moericke (1955*a*) and Müller & Unger (1952) as variation on " alighting flight " induced by weather factors. There is no doubt that aphids flying in the lowest air layers are affected by weather: and that different species show a vertical stratification at times (Broadbent, 1948; Müller & Unger, 1952). But the assessment of individual behaviour with instruments which also measure population changes is a risky procedure (p. 194), especially when the instrument itself exerts such an effect on flight (Moericke, 1955*b*; Eastop, 1955).

Flight itself appears to be more than a mere method of transport : Bruce Johnson (1954) has shown that flight, under experimental conditions and possibly also in nature, is a necessary precursor to a change in behaviour leading to acceptance of a plant. Thus the plant on which the aphid is bred is unacceptable to a new alate aphid (Moericke, 1955*a*, B. Johnson, 1954), and aphids in general prefer young and senescent leaves to mature ones; indeed, host choice may be more a matter of leaf age than of difference in plant species (Kennedy, Ibbotson & Booth, 1950; Kennedy & Booth, 1951). For example, if a new *A. fabae* is allowed a very short first flight of about 20 seconds duration it will accept a plant which before the flight it would reject. The more prolonged the flight, the wider the range of maturity in leaves will the aphid accept, and the longer it will stay on a leaf (B. Johnson, 1955).

It is possible therefore that the acceptability in this respect may be changed by the duration of flight in nature and that aphids descending, e.g., at the end of the day, or at night after a flight, especially if in low light intensity, may accept a wider range of hosts, and even refuel on non-host plants, than they would during the daytime or after short flights. This may have important effects. For the acceptance of a host long enough for young to be deposited marks a turning point in the life of many aphid species. After parturition, in some species the flight muscles are autolysed, the capacity to fly disappearing after a few days; in others autolysis begins before parturition (B. Johnson, 1954). In still other species the flight muscles do not autolyse and the insects are able to fly during several weeks (Haine, 1955).

Thus the acceptability of a host and the flying life of an aphid is intimately associated with the act of flight. There is evidence, however, that the stimulation of handling in the laboratory also affects the relation of flight to host choice and that in nature the effects of flight may be somewhat different from the description given above. Nevertheless, it is clear that host selection and activity, whether by flight or other forms of stimulation, are closely linked.

The acceptability of a host and the state of the aphid and the conditions of light and weather decide if the aphid will make repeated flights from plant to plant once in their vicinity; but little is known of this in the field. How long this kind of flight can continue is conjectural: B. Johnson (1955) produced evidence that the total flight capacity may be lengthened by repeated feeding between flights, and bean aphids are known to visit different plants repeatedly in nature (Müller, 1953). *Myzus persicae* is notoriously restless in this way, and other species deposit young and fly away. Aphids pass out of the accepting state and then require more flight before regaining it (B. Johnson, 1955). Neither is it known if the quality of flight is changed in the early stages of muscle autolysis. These aspects of flight, especially the mechanism of repeated host visitation, are of direct significance to understanding how aphids spread viruses and perhaps why some are more important pests than others. Most work has, however, been done with *A. fabae*, and other species need study along similar lines.

Thus such questions as " How long does an aphid fly ? " or " How far does it travel ? " cannot be answered simply. Neither can such questions be answered comprehensively unless in collective terms as well as in those of individual behaviour. For to variation on the part of an individual at different times is added the variation between different individuals: and for ecological and epidemiological purposes the questions should be reframed thus: " What is the variation in flight duration between different individuals and what proportion of these fly for the different times ? " " What proportion of aphids reach varying distances from a source ? " So, in questions of aerial distributions we must ask " What proportion of an aerial population returns to earth (or near it), once, twice, three times, etc. in a given period during the day ? " These questions cannot yet be answered precisely; but we are proceeding in the direction, in terms of the individual and of the crowd, which I think will enable answers to be given.

DISPERSAL AND THE CONTROL OF BEAN APHIS

A very moderate infestation of *A. fabae* has a serious effect on the yield of field beans: by the time colonies are obvious without close inspection, much damage may be done (Judenko, C. G. Johnson & Taylor, 1952). Moreover, the greatest rate of population increase occurs in the early stages of infestation before colonies are very large (unpublished data).

It is therefore rational to begin control measures before the infestation becomes obvious to a superficial inspection: that is, as soon as the migration has ended (C. G. Johnson (part author), 1952), or with a persistent insecticide before this. Spectacular contro has been carried out along these lines by Way, Smith & Potter (1954).

The duration of migration is itself very variable, and may occupy 1 week or 4 or 5 weeks in different years : and with the known effect of wind on dispersal and the commonly observed " edge infestations " on beans it was worthwhile to attempt to correlate wind direction with infestation pattern (C. G. Johnson, 1950; Taylor & C. G. Johnson, 1954). We found that often the windward edges bore a heavier deposition of migrants; and often also a heavier colony growth which it is plausible to suppose was due to the delivery of more migrants on the windward side. But there are sometimes more colonies near trees and hedges irrespective of wind direction and number of migrants deposited. We have found recently that very small temperature rises above the developmental threshold, especially between 16° and 21° C., increase the rate of development by more than twice compared with a similar temperature rise above 21° C. : it now appears to be worth while to study the rate of colony growth in relation to microclimates in sheltered compared with exposed places in the crop as well as to deposition of migrants.

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THE HATCHING OF CYST-FORMING NEMATODES

By

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The genus *Heterodera* contains a number of species of cyst-forming plant-parasitic nematodes. They are highly specialized morphologically and biologically, and one of their adaptations to parasitism is their selective response to substances which are produced by the roots of host plants and which are generally referred to as root diffusates. Before considering the work of the Nematology Department on this subject it is opportune to summarize the life history of a typical member of this genus. Soil infested with *Heterodera* spp. contains round or lemon-shaped bodies known as cysts about ½ mm. in diameter and brown in colour; these cysts may contain up to 700 eggs, each of which may contain a larva. In the absence of host plants larvae emerge only slowly—in the case of the potato-root eelworm *Heterodera rostochiensis* Woll. about 40–50 per cent in one year; the cysts can therefore remain infestive for several years. When a host plant is present diffusates produced by the growing roots stimulate the larvae to emerge rapidly—with the potato-root eelworm up to 80 per cent of the contained larvae emerge from cysts in the vicinity of plant roots in the first few weeks of the plant's life. Following penetration of the roots, development of the larvae results in the production of a new generation of flask-shaped females which protrude from the roots as white, rounded bodies containing large numbers of eggs. They lose their white colour and become brown, the body hardens and dies, the dead body acts as a protective envelope for the viable eggs and later becomes detached from the roots to lie freely in the soil. This is the cyst stage referred to above.

Hatching from beet-eelworm cysts (*Heterodera schachtii* Schmidt) was shown by early German workers to be stimulated by diffusates produced by the roots of host plants. Triffitt (1930) investigated this in detail. Working with *H. rostochiensis*, she concluded that potato roots would produce a diffusate capable of stimulating larval emergence only as long as root growth was actively occurring; the stimulatory factor was heat resistant and active in high dilutions; its breakdown was rapid under non-sterile conditions. Triffitt also confirmed the findings of Morgan (1925) that the diffusate from mustard roots was antagonistic to potato-root diffusate and did not stimulate potato-root eelworm; the substance was present in shoots and, unlike potato diffusate, was comparatively stable under non-sterile conditions. She also reported a dormancy period during the winter when larval emergence was extremely slow. In later papers (1932, 1934) Triffitt presented data showing that certain grasses produced diffusates which stimulated the hatching of potato-root eelworm. Franklin (1937) detected the same effects with diffusates of white mustard and yellow maize.

Our present understanding of the problem throws considerable doubt on the reliability of a great deal of this work, which was based on the assumption, now known to be erroneous, that all cyst-forming species of *Heterodera* were biological strains of *Heterodera schachtii*; the very high level of variability exhibited by eelworm cysts, and the absence of sound statistical design, make it difficult to assess the validity of the findings. The main conclusions to be drawn appear to be that root diffusates can induce the emergence of larvae from cysts; their action is specific, i.e., a given species of eelworms can only be stimulated by diffusates from certain plants.

Work carried out in the Nematology Department on larval emergence of *Heterodera rostochiensis* can conveniently be divided into three main sections :

(*a*) The development of satisfactory techniques for the conduct of hatching tests.
(*b*) An examination of the factors influencing hatching in the laboratory and in the field.
(*c*) An attempt to elucidate the chemical nature of the active principle in potato-root diffusate.

Techniques

When hatching tests were commenced it was found that a number of factors rendered their conduct very difficult : the number of eggs per cyst within any population was very variable, which resulted in tests being subject to large and unpredictable errors : cysts from different populations differed considerably, both in egg content and also in their response to hatching stimuli, so that it was difficult to obtain results capable of generalized application. Nothing was known about the " quality " of the diffusate used, since the method for its collection was not amenable to rigid control, and the chemical constitution of the active factor was unknown.

Preliminary investigations involved isolating the larvae hatched from single cysts, for this a new slide was designed (Fenwick, 1943) as a refinement of the single-cyst techniques of Gemmell (1940) and Ellenby (1943). Using this slide, 50 cysts could be accommodated individually in glass cups on a 6 × 6-cm. glass plate. Using cysts from a number of populations and exposing them individually to diffusate drawn from a single bulk stock, it was possible to investigate the variability exhibited within and between different populations. The results of these investigations (Fenwick, 1949) showed that if batches of 100 cysts were used as units in hatching tests a basic minimum error of ±10 per cent was to be expected.

The use of units of this magnitude introduced new problems of technique, the most important of which was the counting of the thousands of larvae produced. Two modifications (Fenwick, 1951*c*; Peters, 1952) of the McMaster slide (Gordon & Whitlock, 1939) enabled this to be done by dilution. To overcome the tedium and labour in setting up replicate batches of cysts, a weighing technique (Fenwick & Reid, 1952) and later a sucking technique (Hesling, 1952) were developed. The use of either of these methods in conjunction with the mechanical purification methods developed by Hesling (as yet unpublished) have made it possible to conduct hatching tests on a larger scale than could previously have been contemplated.

The investigations of Elizabeth Reid (afterwards Widdowson) into problems of production and storage of root diffusates have led to a more standard and reliable product which can be preserved for several months with little loss of activity.

A difficulty associated with hatching tests has been the fact that larval emergence in the laboratory is a protracted business occupying up to 3 or 4 months. Preliminary investigations into the form of the hatching curve (Fenwick, 1950*a*) showed it to be sigmoidal in character when hatch was plotted against log time. Use was made of this relationship to forecast, at the end of 10–12 days, what the final hatch would be (Fenwick, 1951*b*). Although this shortened the total duration of a test, the need for daily counts during this period increased rather than decreased the total labour required on a test. Later work has shown this labour to be unnecessary, and it is now standard practice to run a test for 21 days, the hatched larvae being counted at 7 and 21 days. By this method 85 per cent of the hatchable larvae are recovered.

In any hatching tests, two classes of larvae are recognizable—those which hatch and those which do not. The former are counted when they emerge, but the latter are frequently of equal importance, and it is often necessary to estimate them quantitatively. Two techniques for estimating the unhatched larval content of cysts have been developed (Reid, 1952 and 1955) and are of great importance in potato-root eelworm research; they form the basis of the egg-hatching experiments referred to later, and are in general use for infestivity investigations outside Rothamsted.

As a result of the development of the above techniques it has been possible to lay down standard hatching procedures giving results of predictable accuracy: even so, a very high degree of replication is necessary if a reasonable degree of precision is to be obtained. In hatching tests conducted by the writer over several years, using weighed batches of 150–200 cysts in five-fold replication, the overall errors have been of the order of ±20 per cent. This has been shown to be due, primarily, to the variability in the egg content of individual cysts. A new technique has been developed in which eggs, after removal from the cysts, are exposed to the action of diffusates. There is every reason to believe that the errors resulting from cyst variability can be significantly reduced by this method, with a substantial saving in material and effort.

The " dormancy period " experienced by Triffitt, when hatching was very slow, has been investigated, and techniques have been described (Fenwick & Reid, 1953) for the storage of cysts, which have enabled hatching tests to be conducted throughout the year, with no apparent diminution of hatching in winter.

The techniques so far described have all been concerned with measuring and reducing the errors resulting from the variability in egg content of *Heterodera* cysts. Variations in potency of natural diffusates are of equal importance, and a technique has been developed for standardizing a given sample (Fenwick, 1952*b*). If a sample of diffusate be diluted in a logarithmic series, larval emergence in each successive sample decreases in a linear manner. It was found possible, by plotting a curve, to calculate a

" threshold " value of concentration beyond which a sample of diffusate was inactive. A strength of 1 arbitrary unit of concentration was ascribed to this " threshold ", and original concentration was expressed in terms of it—i.e., if the " threshold " for a sample corresponded to a 1/1,000 dilution, then the strength of the original sample was 1,000 arbitrary units. In practice a new term was used—the L.A. value (the logarithm of the concentration of the hatching factor)—which was proportional to the hatching ability of a given sample of diffusate. Using this technique, it has been possible to secure by dilution diffusates of known L.A. values and to measure quantitatively root-diffusate production and breakdown.

All the experiments so far described have been carried out on apparently " normal " cysts as recovered from naturally infested soil, i.e., they have not been treated with nematicidal fumigants. As far as the writer is aware, the relatonships so far mentioned in this review have never been tested on cysts exposed to nematicides. This point is worthy of emphasis, since many workers have sought to utilize hatching responses as a method of estimating kill following nematicidal treatment. In the absence of data showing the effect of chemical pretreatment on the parameters of the hatching dilution curves, interpretation of these workers' data is at most conjectural. Even if the same or new relationships for hatching can be applied to cysts treated with nematicides, the " hatchability " of larvae may not be synonymous with their " viability ". To regard the results of hatching tests as more than a mere indication of viability is an unjustified assumption. A thorough investigation into the relationship of hatchability to viability is an essential preliminary to the use of hatching tests in nematicidal work.

Larval emergence in the laboratory and in the field

Diffusate for hatching tests on *H. rostochiensis* is usually obtained from potatoes grown in 6-inch pots of 3 : 1 loam and sand, and knowledge is now available of some of the factors influencing its production. Elizabeth Reid has carried out experiments on the relationship of the age and variety of potato and tomato plants on root-diffusate production, and has investigated the effect of infestation with potato-root eelworm on root-diffusate production. Her work indicates that root-diffusate production is closely correlated with rate of increase of root growth measured by weight : total root weight plays a subsidiary but significant part. In the case of potatoes, diffusate production is at a maximum 5–6 weeks after planting, and this is the period of maximum rate of growth under experimental conditions. A large number of potato varieties has been tested, but there is little evidence of marked differences in the potency of the diffusates produced. Comparisons between potatoes and tomatoes showed that in the early stages of growth, diffusates from the latter were less active, but in older plants diffusate production in both was comparable; as potatoes require less attention than tomatoes, they are more suitable plants for root-diffusate production. Increased root-diffusate production resulted when potatoes were exposed to a low to medium rate of infestation with eelworm, which stimulated root proliferation. Heavy attacks stunted root growth and reduced the production of diffusate. Root-diffusate production appeared to be a function of growth. Elizabeth

Reid has shown that root diffusate is produced equally well in soil, in sand with the addition of a plant nutrient or in sand watered only with distilled water; in the last case, however, production was not maintained for so long.

Root-diffusate production by plants other than potatoes and tomatoes has received attention. J. J. Hesling has repeated the work of Triffitt and of Franklin on the effects on *H. rostochiensis* of root diffusates of Gramineae. Fourteen species of grasses and five varieties of cereals were tested. Diffusates collected after three weeks growth did not stimulate the hatching of *H. rostochiensis*. He also compared diffusates produced by *Solanum andigenum* Juz. and Buk., *Solanum demissum* Lindl. and potato. All three species produced active diffusates, but as no account was taken of root size or growth rates comparisons among them were difficult.

Investigations by C. C. Doncaster showed that the nightshade *Solanum nigrum* L., which is not a host of potato-root eelworm, although it can be invaded, produced a very active diffusate. In the presence of even light infestations, diffusate production in young plants was reduced, but this effect was not so marked with older plants. Experiments on trap-cropping with this plant gave disappointing results.

Doncaster (1955) has also worked on the hatching responses of *Heterodera cruciferae* Franklin by diffusates produced by sprouts, swedes, rape kale and mustard. They are all active, although mustard is less so than the other three. There is no evidence that the addition of mustard diffusate to the others has any inhibitory effect. He finds that the hatching curves for these diffusates are sigmoidal and that their dilution curves are similar to those for potato-root diffusate.

Attempts by J. J. Hesling to obtain a diffusate which stimulates the hatching of *Heterodera major* (O. Schmidt) have been unsuccessful.

Experiments on the factors influencing the hatching of *H. rostochiensis* in the laboratory have been carried out (Fenwick, 1951*a*). Presoaking of dry cysts for 7–12 days in tap water before exposure to root diffusate increases the rate of hatching, but has no effect on the total number of larvae emerging from replicate batches of cysts. A constant temperature of 25° C. appears to be the optimum, but 30° C. inhibits hatching. Within wide limits larval emergence is not influenced by the volume of diffusate used nor by the number of cysts in that volume. Direct sunlight inhibits hatching, and this effect is permanent; cysts having been exposed to it, still do not hatch when transferred to the dark; diffused light is without effect. Hatching is unaffected by pH between 3·2 and 8·1. Hesling has found that if cysts of different sizes are exposed to potato-root diffusate there is a tendency for larvae to emerge from smaller cysts more readily than from the larger.

The hatch in water varies between the different species of *Heterodera*. In *H. rostochiensis* emergence is very low, but considerable spontaneous hatching can occur in the case of *H. cruciferae*, *H. trifolii* and *H. schachtii*; *H. göttingiana* and *H. major* do not normally hatch in water. The hatching of *H. major* has been investigated by Hesling, who found that it occurred in spring and summer as a result of a rise in temperature, the effect of which lasted

6–10 days. Hatching from the cysts of *H. major* was inhibited by drying. When cysts were exposed to different relative humidities he found that those exposed to R.H. 98–100 per cent " hatched " equally well as cysts which had not been dried. Exposure to R.H. 87 per cent and lower inhibited hatching for a considerable time.

Larval emergence in soil and sand has not been investigated in such detail as it has in fluid media. J. J. Hesling found that hatching of *H. rostochiensis* in sand saturated with root diffusate was very similar to that observed in watch-glasses—the hatching curve was sigmoidal and its parameters did not differ substantially from those in fluid media. Other work on this subject has been done by Elizabeth Reid. She used shallow seed pans 12 inches in diameter filled with damp sand which had been uniformly infested with potato-root eelworm cysts. Known volumes of root diffusate were added to the centre of each pan twice daily for 8 weeks. After this time the larval content of cysts near the application point had fallen by 80 per cent, compared with a fall of 40 per cent near the perimeter.

Fenwick (1950*b*) investigated the effect of root diffusates from different varieties of potatoes in natural infestations of cysts on three different soils. In the absence of diffusate larval emergence over a season was about 50 per cent : in the presence of diffusate, 84 per cent of the larvae emerged. There was evidence of differences in response to diffusates from different varieties of potato and in the response of cysts from different localities to a single diffusate.

Hatching of *H. major* in soil under natural conditions has been shown by Hesling to occur at a steady rate from March to July during which time about 50 per cent of the cyst contents emerge. After July, hatching is considerably reduced.

Interesting results have been obtained by comparing hatching from free eggs of *H. rostochiensis* with eggs within their cysts. The general relationships were similar in both cases : the hatching curve was sigmoidal and the dilution curve was linear, although in general the rate was slightly faster in the case of free egg hatching. An interesting effect has been observed as a result of presoaking experiments. Eggs from cysts presoaked in water for 7 days hatched faster in root diffusate than those from dry cysts. If eggs from dry cysts were soaked, soaking appeared to inhibit subsequent hatching. This was the case whether particles of cyst wall were present during soaking or not—merely squashing cysts before soaking was sufficient to inhibit hatching of eggs. Soaking of whole cysts for as little as 1 hour counteracted this effect. No convincing hypothesis has been advanced to explain this curious result.

A factor which must influence larval emergence in soil is the degree of persistence of diffusate. The stability of potato-root diffusate under storage conditions has been investigated by Elizabeth Reid : she has confirmed Triffitt's findings that at room temperature it breaks down fairly rapidly; at 0–5° C. no appreciable loss in activity occurs over 12 months.

Fenwick has investigated its breakdown in soil. In a naturally infested heavy loam, breakdown was very rapid—90 per cent of the active principle was lost in 4–6 days, and the effect of a single application on the egg content of cysts was very small. When root diffusate was applied to horticultural peat, clay, sand and gravel its breakdown in peat was less rapid than in the other three media. When repeated applications of root diffusate were made to soil the breakdown of later applications was more rapid than of the earlier. Breakdown in partially sterlized soil was less rapid than in unsterilized soil. The author believes that in soil, breakdown is due to the action of micro-organisms which utilise root diffusate as a substrate. The results of these experiments cast considerable doubt on the practicability of using root diffusate as a method of eelworm control; it would appear unlikely that root diffusate can persist in soil long enough to have any appreciable effect in causing larvae to hatch in the absence of a host plant.

Chemical investigations

In 1951 a project for research into the chemical nature of the potato-root eelworm hatching factor was financed by the Agricultural Research Council to be carried out jointly at Reading, Cambridge and Rothamsted. At Reading, Professor R. H. Stoughton grew potatoes in order to produce the active diffusate; at Cambridge, Professor Sir A. R. Todd and his collaborators worked on the analysis of the active principle; at Rothamsted, the Nematology Department undertook the assay of "fractions" produced at Cambridge.

Preliminary isolation of the factor (Johnson, 1952) was accomplished by adsorption of the active principle from crude leachates on animal charcoal which was subsequently eluted with aqueous acetone. The hatching factor was found to be relatively stable between pH 2 and 7, but was rapidly deactivated at pH 8 and over. It was acidic, of low molecular weight and probably contained a lactone group. Treatment of the active brucine salt with acid followed by exaustive extraction with ether gave an acidic resin, active at concentrations of 1×10^{-7} to 1×10^{-8} and the name "eclepic acid" was proposed. Because of the heavy losses of active principle involved in this process, physical methods of purification were examined. Extraction of the crude concentrate with ether proved useful, as also did partition chromatography in silica gel buffered at pH 6. Titration of the product indicated an equivalent of 250–290; there was evidence of at least one double bond in the compound, and an approximate formula of $C_{19}H_{26}O_8$ was proposed.

Armitage (1955) attempted concentration by vacuum distillation. Phenol extraction of the product resulted in negligible recovery of the active principle. He then resorted to ion-exchange techniques. After preliminary passage through a cation-exchange resin (Zeokarb 225) the activity was retained on an anion-exchange column (Deacidite FF) and recovered from this by elution with $3N$–HCl: the product had considerable activity, but phenol extraction of it resulted in considerable losses. Paper-strip chromatography of the organic acid fraction using a *n*-butanol–water–formic acid developer disclosed six acids. All attempts to obtain a solid derivative failed, as also did attempts to separate the water-soluble organic acids, whether by counter-current distribution, ion-exchange chromatography or partition chromatography.

After Armitage's departure from Cambridge in August 1955 the joint work with Cambridge and Reading ceased, but a series of joint small-scale experiments was commenced by D. W. Fenwick and G. N. Wiltshire at Rothamsted. As a result of cation exchange by charcoal adsorption and acetone elution, these workers reduced the dry weight of substances to 0·5 per cent of the original, with only a small loss of the active factor; this corresponded to a purification factor of ×184. Further work on this is in progress, but is not as yet far enough advanced for comment.

This review is a summary of the work carried out in the Nematology Department, Rothamsted. Valuable research is being carried on at several other centres, but there is not space to describe them in this review.

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