ROTHAMSTED RESEARCH

Report for 1982 - Part 1

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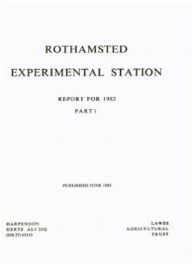

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T. Lewis (1983) *Entomology Department ;* Report For 1982 - Part 1, pp 91 - 108 **- DOI: https://doi.org/10.23637/ERADOC-1-129**

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Introduction

Impressive progress on several established projects has been achieved during the year and opportunities taken to begin new studies in response to changing emphases and requirements of agriculture.

With help from several pesticide firms, a regional comparison of cereal aphid populations in Rothamsted Insect Survey traps and nearby fields was shown to be feasible, and

https://doi.org/10.23637/ERADOC-1-129

the experience gained will be used to validate further the suction trapping network. The first radar equipment, which will eventually complement these traps to provide early warning of pest infestation, was purchased but it will take several years to build, test and exploit the system envisaged. Meanwhile, the basic studies on aphid distribution have continued, and interesting results from the unique studies on the variability of cereal aphid populations throughout the country have begun to appear.

In response to the increasing hectarage of the oilseed rape crop, new work on its pests has begun; likewise, the concern for sporadic but locally serious damage to cereals and potatoes by slugs is being met by a determined approach to develop new molluscicides. The prospects of developing fungal pathogens to be used directly as insecticides have been enhanced by studies on spore penetration of insect cuticle and methods of field dissemination. Further pheromonal studies on bees have yielded two extremely useful 'tools' for the chemical management of colonies, and with the help of outside organizations the pea moth monitoring system has been developed to provide a more reliable service to individual farmers.

The new and detailed basic studies on elemental analysis of insects are indicating a valuable potential for distinguishing populations of insects from different geographical origins, and, if successful, the techniques might be developed for wider uses such as identifying the sources of animal and plant virus vectors.

The earthworm work on farm waste degradation and protein production has gained momentum and scientific depth with the development of larger scale systems of production, new microbiological studies funded by MAFF and further involvement with ARC institutes and university departments.

Overall, one can point to a year of vigorous and productive research with the dual satisfaction of having provided new and usable techniques for current agriculture, plus an investment in long-term basic knowledge.

Cereal aphid studies

Regional distribution. 1982 saw the introduction of the Rothamsted Insect Survey Cereal Aphid Monitoring Scheme (RISCAMS) which was set up primarily to investigate the extent of variation in aphid numbers between fields within and between different regions of the country. Previous correlations between crop populations and catches of cereal aphids in the nearest suction trap were significant in some years, e.g. 1977 and 1979, but poor in others, e.g. 1980 and 1981, due to large differences between fields. RISCAMS was designed to examine some of the factors contributing to these differences.

As a pilot study, one hundred wheat fields were sampled, 37 in Hertfordshire and Bedfordshire, 30 in Suffolk and Norfolk, and 33 in Essex. All the fields lay within a 32 km radius of the traps at Rothamsted, Brooms Barn and Writtle respectively. Only wheat fields were surveyed, since this crop is usually the only one treated with aphicide in the summer.

Monitoring was carried out by three temporary staff trained in aphid identification, sampling procedures and data recording, and based at Rothamsted, Brooms Barn and Writtle. Each field was examined once a week between 7 June and 2 July.

Aphid populations were estimated by counting the proportion of tillers infested rather than by counting the actual number of aphids present. This enabled many fields to be sampled in a short time and the percentage infestation was then calibrated with actual density using previously established regression equations. The cultural and fertilizer histories of each field were also recorded to try to correlate differences in aphid abundance with agricultural practice. The record sheets were posted daily to Rothamsted where weekly summaries were produced and sent by telex to the sponsors of the project, ICI, Shell and Bayer UK and also to MAFF at the Harpenden Laboratory.

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Aphid numbers were low in England in 1982 with most fields requiring no insecticidal treatment. As a result, no obvious effects of agricultural inputs on aphid numbers were detected, although the data are still being analysed. There were small differences between regions which will be investigated in future years.

This preliminary study showed that such a survey was feasible and there was great interest in the results, especially from participating farmers. It is hoped to extend the project to more trap sites, but this is dependent on further financial sponsorship. Thanks are due to ICI, Shell and Bayer UK for their interest and initial support. (Dewar)

Genetic variability of populations. During 1981 and 1982, work continued on the genetic variability of *Sitobion avenae* (F.) populations using isoenzyme markers, emphasis moving towards a study of biochemical polymorphism within and between field populations (*Rothamsted Report for 1979*, Part 1, 85-86; *Rothamsted Report for 1980*, Part 1, 95-96). In addition, because of continuing uncertainty encountered in visual separation of (anholocyclic) *S. avenae* from the morphologically very similar (holocyclic) *S. fragariae* (Walker), populations of both were compared electrophoretically to determine the degree of genetic divergence between them. Earlier work had shown the species to differ in the number of peptidase isoenzymes (*Rothamsted Report for 1979*, Part 1, 85-86).

In 1981, *Sitobion* populations were sampled in June and July at various sites in southern England. Random samples of about 1000 individuals of *S. avenae* were collected from wheat at four main sites (Long Ashton, Bristol; Fordingbridge, Hants; Brooms Barn, Suffolk; Norwich, Norfolk), and about 1000 *S. fragariae* were collected from *Dactylis glomerata* at Salcey Forest, Northants. Smaller samples of *Sitobion* (<200) were collected at other sites (including Rothamsted) from a variety of hosts, and aerial *S. avenae* populations were sampled at Rothamsted using 1·2 m suction traps in order to compare their variability with that of established field populations. All areas sampled covered <10 ha. Prior to electrophoresis, aphids were stored deep frozen at $-25°C$.

Sitobion spp from the large samples were surveyed electrophoretically using five enzymes (representing 13 gene loci), known from previous experiments to show polymorphic bands in *S. avenae*. These enzymes were esterase (EST), glutamate-oxaloacetate transaminase (GOT), peptidase (PEP), phosphatase (PHOS) and peroxidase (POD). Usually, about 200 aphids were run per enzyme system. With *S. fragariae* one enzyme (POD) was not detected by staining after prolonged frozen storage. The smaller samples were examined only for esterases.

For some enzyme loci (e.g. EST-1, PEP-4 & 5, PHOS-2 & 3, POD), there were clear differences in allele frequency between sites, whereas others were either largely (e.g. EST-2,4,6 & 7, PEP-1, PHOS-1) or completely (e.g. GOT) invariant. χ^2 contingency tests on data for these loci showed most pair-wise comparisons of populations to be significantly different ($P=0·05$), indicating that the *S. avenae* populations consisted of numerous clones, and were not genetically identical.

To examine this point further, the data were compared using Nei's coefficients of genetic identity (I) and distance (D). For some polymorphic loci (e.g. POD), I values varied widely between sites (i.e. $0·17-0·99$), whilst others (e.g. PHOS-3) less so (i.e. $0·62-0·98$) (an I value of 1 and D value ($-\log_e I$) of 0 = no genetic divergence). On summating I values for all 13 loci sampled per population, Fordingbridge proved to be the most genetically divergent of the large *S. avenae* populations examined, reflecting either random genetic drift or sampling effects. The mean I and D values for population pair-wise comparisons were $0·939 \pm 0·038$ and $0·063 \pm 0·041$ respectively. Such small D values are typical for mobile insect populations of the same species.

In contrast, I and D values between an *S. avenae* (Long Ashton) and *S. fragariae*

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population (Salcey) were 0·35 and 1·05 respectively, supporting the view that they are in fact well-defined species.

The survey of esterases produced a variety of results. Firstly, EST-1 (the only locus examined in *S. avenae* commonly showing heterozygotes) produced a generally high level of heterozygosity (H) for all *S. avenae* populations. There were distinct differences in the frequency of heterozygotes between some sites; values ranged between 45% (Long Ashton) to 91% (Rothamsted) (mean 70·6% for seven populations). Average H values for aerial and field populations collected at Rothamsted were very similar, 86·4 and 91·4% respectively. Such values are in excess of average H values per locus reported for a wide range of sexually reproducing animals, and may result from selection at this locus in the largely parthenogenetic *S. avenae*. This conclusion is further supported by the finding that the number of EST-1 heterozygotes were, for most sites, in excess of that expected from a sexually reproducing population in Hardy–Weinberg equilibrium (average χ^2 probability for eight populations $P<0{\cdot}001$). In contrast, H values for the holocyclic *S. fragariae* were much lower for two populations sampled ($H<22\%$) and did not deviate from H-W expectations.

Secondly, of all *S. avenae* populations sampled, no intense staining esterase bands, such as are known to be associated with insecticide resistance in other pest aphid species, notably *Myzus persicae*, were observed (*Rothamsted Report for 1977*, Part 1, 139-140). This agrees with findings of Devonshire *et al.* (*Rothamsted Report for 1979*, Part 1, 116).

In 1982, large *S. avenae* populations were again sampled from wheat at Long Ashton and Norwich, and locally at several sites around Rothamsted. A number of populations at geographically widespread sites in Spain were also surveyed. The aim of these surveys was (a) to see if allele frequencies vary temporally as well as spatially, (b) whether there is a similar degree of genetic divergence locally as has been observed between more distant populations, and (c) to monitor genetic divergence between geographically very distant populations. The results are still being analysed. (Loxdale)

Oilseed rape pests

During the last decade autumn oilseed rape (*Brassica napus*) has largely replaced spring sown varieties to become an increasingly popular and profitable break-crop in most cereal growing areas of England. In 1982, it covered about 124 000 ha and a further increase is anticipated for 1983. This dramatic increase in crop area, with possibly further changes in varieties and cultural practices, stimulated re-examination of potential crop losses due to insect pests, particularly the pod midge (*Dasyneura brassicae*). Observations were made, therefore, on some insect pests and yield components of oilseed rape grown on four commercial farms and at Rothamsted.

In the laboratory, the ratio of male to female pod midge emerging from larvae collected from individual stems taken from fields in June differed greatly. This suggests that like other species of Cecidomyiidae individual female pod midges may produce unisex offspring.

In rearing cages kept outdoors possibly four generations of *D. brassicae* emerged during May-July. A similar impression of the insect's phenology was obtained from catches in yellow water traps in a rape crop during May–August; assuming that the first two generations overlapped in May–June, a third appeared in July and a fourth in early August.

The most common insect pests in water traps during flowering in May were the pollen beetle (*Meligethes aeneus*) and the seed-weevil (*Ceuthorynchus assimilis*), with fewer thereafter until July–early August when the next generation emerged. Few stem weevil (*C. quadridens*) were caught in May–August. Relatively few *D. brassicae* were trapped in

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May–June, but their numbers increased to peaks in both July and August, during and after harvest; traps sited within and at the edge of crops caught similar numbers.

Pod infestations on all farms confirmed that oviposition by *D. brassicae* depends on the availability of feeding and ovipositional punctures made by *C. assimilis*. Large larval infestations by both species occurred on two farms just after flowering in early June but by harvest the proportion of pods infested was small on all five farms.

Similar numbers of auxillary racemes/plant, intact pods/raceme and pods/plant were found on the four commercial farms where yields from Jet Neuf were also similar (approximately 2·7 t ha^{-1}). As expected, all these yield components were smaller in the Rothamsted plots (var. Primor) because of bird damage. Neither shattered pods, which may be caused by *D. brassicae*, disease and birds, nor blind pedicels, variably attributed to *M. aeneus*, could be used in 1982 to ascribe with certainty the cause of crop loss. Undoubtedly small birds were responsible for much of the crop loss due to pod shattering, and insect attack was almost certainly slight and early enough for the rape plants to produce compensatory growth. (Dean)

Pheromonal studies

Nasonov pheromone of honeybees. The Nasonov gland of the worker honeybee discharges into a groove in the seventh abdominal tergite and is exposed by flexing the tip of the abdomen. The pheromone it secretes contains geraniol, nerolic and geranic acids, (*E*)- and (*Z*)-citral, (*E*,*E*)-farnesol and nerol.

When bees regain their hive entrance after being temporarily lost they often expose their Nasonov glands and disperse the pheromone by fanning; this helps other bees to find their hive. The presence of Nasonov pheromone at the hive entrance stimulates returning foragers to release Nasonov pheromone themselves reinforcing the homing signal. The effect of individual components in inducing Nasonov exposure was tested; synthetic (*E*)-citral, geraniol, nerolic acid and geranic acid components stimulated exposure and the omission of any one of these from the synthetic mixture diminished it. By contrast with the other components, (*Z*)-citral, (*E*,*E*)-farnesol and nerol had little effect on Nasonov exposure when tested singly and omission of either farnesol or nerol from the Nasonov mixture increased the attractiveness of those that remained.

After swarming bees have left their hives they cluster nearby before flying to a new home. The first workers to settle release Nasonov pheromone which attracts the queen and still airborne workers to cluster with them. Use was made of this behaviour to develop a bioassay to test the response of artificial swarms to various components of Nasonov pheromone. Geraniol, (*E*)-citral and nerolic acid proved the most effective of the seven components for inducing clustering. The presence of (*Z*)-citral with (*E*)-citral did not diminish clustering and sometimes increased it. Farnesol and nerol, if anything, tended to cause clustering bees to cease scenting while the presence of the other gland components encouraged exposure to continue.

As a result of these findings a practical and effective mixture for attracting honeybees to cluster had been developed; it consists of a 1:1:1 mixture of geraniol, nerolic acid and (*E*)-citral (in readily available forms which contain isomeric impurities) but no farnesol or nerol.

Swarms were much more attracted to empty hives containing the mixture in polyethylene vials than to unbaited hives both in confined and free-flying conditions. Because beekeepers cannot control swarming, the lure could have widespread application, especially in making beekeeping more profitable in parts of the world where migration and absconding of colonies is common.

This same Nasonov pheromone lure also stimulates bees to consume water, pollen

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substitute and sucrose syrup offered inside their hives, thus helping to build up colonies to be used for honey production and pollination in spring and early summer.

Attracting bees to water inside the hive can be especially valuable early in the year when cold weather may make foraging hazardous. The standard lures could also be used to condition bees to collect water provided by beekeepers near to hives, so that the bees would be less likely to create a nuisance by visiting water sources used by man (e.g. taps, swimming pools, ornamental ponds).

The Nasonov lures are also effective, especially in association with the queen pheromone component (*E*)-9-oxo-2-decenoic acid, in inducing honeybees to enter traps, so they can be removed from sites (glasshouses, food processing factories) where their presence is undesirable. (Free and Ferguson, with Pickett and Smith, Insecticides and Fungicides Department)

Alarm pheromones of honeybees. When a honeybee stings an intruder, or is alerted to do so, it releases alarm pheromones that mark the enemy and direct the attack of other defending bees towards it. Several compounds from the sting have now been identified, but only 2-nonanol, iso-pentyl acetate and n-butyl acetate from the sting gland and 2-heptanone from the mandibular glands have been found to elicit attack and release stinging. Three compounds, iso-pentyl acetate, n-octyl acetate and 2-heptanone repel clustering bees and diminish their Nasonov exposure.

Colonies can become adapted to alarm pheromones introduced into the hive, with the result that their aggressiveness is diminished. This should have important practical applications by helping beekeepers to manipulate their more ferocious colonies. (Free, with Mr Bader Al-Saad and Dr P. E. Howse, Southampton University)

Recent experiments have shown that high concentrations of the two honeybee alarm pheromones, iso-pentyl acetate and 2-heptanone, discourage foraging on sugar syrup, sunflowers, field beans, and oilseed rape. It may be possible to develop formulations that can be applied to flowering crops immediately before an insecticidal treatment, thereby diminishing bee mortality. (Free and Ferguson, with Pickett and Smith, Insecticides and Fungicides Department)

Properties and dispersal of pea moth pheromone and field behaviour. The synthetic pheromone (*E*,*E*)-8,10-dodecadien-1-yl acetate (*E*,*E*8,10-12:Ac) degrades in the presence of sunlight to produce one or more compounds which are inhibitory to male pea moths when tested in field traps. During field tests of fractions of degraded *E*,*E*8,10-12:Ac separated by column chromatography, the inhibitory properties of certain fractions were demonstrated. Traps containing the attractant and an inhibitory fraction failed to catch moths; moreover, moths were repelled from an attractive trap by temporarily placing an inhibitory fraction immediately adjacent to the trap. When the inhibitory fraction was removed 10 m cross-wind moths approached the attractive trap once more, after a short delay. (Wall, with Greenway, Insecticides and Fungicides Department)

The formation of this inhibitor can be prevented by formulating the attractant with an antioxidant, to produce a slow release formulation which remains attractive in the field for at least 60 days (*Rothamsted Report for 1981*, Part 1, 132). Collaborative experiments with INRA, France, have shown that previous discrepancies between field results obtained in the UK and France were caused by this degradation process upsetting results in France (see Insecticides and Fungicides Report p. 128 for chemical details). (Wall, with Greenway, Insecticides and Fungicides Department, and Dr R. Bournoville and Dr J. Einhorn, INRA, France)

The way in which insects fly towards distant point sources of wind-borne odour over open ground has been reappraised in the light of meteorological and smoke-trial evidence

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that the wind carries the odour from the source in straight lines. It is suggested that insects do not fly along the meandering plume, but fly upwind, and thus directly towards the source, when stimulated by the odour. Thus, their tracks will often be across the longitudinal axis of the meandering plume. (Wall, with Perry, Statistics Department and Dr C. T. David, Dr A. R. Ludlow and Professor J. S. Kennedy, Imperial College, Silwood Park)

The dispersal of an odour from a point source within, say, a cereal crop differs from that over open ground (*Rothamsted Report for 1979*, Part 1, 162). In a cereal crop the discrete plume probably exists for only a short distance downwind of the source; further downwind a continuous field of pheromone is formed. Diffusion equations best describe dispersal in this situation. Mathematical models of pheromone dispersal and pea moth responses in a crop are being used to test hypotheses of moth behaviour (see Statistics Report, p. 284). Parameters for these models have been derived from work on (i) trap interaction, and (ii) the uptake and release of pheromone from vegetation. (i) Work on trap interactions has been extended to include an investigation into the systematic changes in the profile of catches in lines of traps within any daily activity period; experiments indicate that these changes occur simultaneously in separate lines of traps within one field. (ii) The discovery that male moths can use a 'trail' of pheromone adsorbed on to vegetation to find their way to the source of that pheromone has thrown new light on our understanding of their orientation behaviour. The fact that a trap containing 100 μg *E,E*8,10-12:Ac placed in a wheat field for just 30 min can result in moth attraction and sexual activity around that site for 2–3 h after removal of the trap demonstrates the importance of the vegetation in the communication system. Interestingly, moth activity increases sharply during the first few minutes after trap removal, possibly because moths which have been attracted near to the trap but not caught then respond to the lower dose coming from the vegetation. Further experiments have shown similar results in pea crops and over artificial plastic 'grass'; even with the latter the moths are able to find the exact previous position of the trap.

One of the main conclusions of the work, and assumptions of the mathematical model, has been the long range (several hundred m) attraction of male pea moths to traps containing 100 μg *E,E*8,10-12:Ac. The results of a mark and recapture experiment verify this conclusion. Individually marked moths were released downwind of a trap at two distances (160 m and 500 m); their behaviour on release, the number recaptured at the trap and the time taken for recapture were recorded. Moths released at both distances showed clear signs of responding to the odour from the trap; 8% of those released at 160 m and 4% of those at 500 m were recaptured within 3 h, and two moths released at 160 m were recovered after 1 min 55 s and 3 min 25 s (equivalent to a minimum average ground speed of 0·8–1·4 m s^{-1}). (Wall, with Perry, Statistics Department)

Improved monitoring for moth pests. Following a suggestion by Rothamsted staff that on-site monitoring of pea moth in dried pea crops may be improved by specialist interpretation of trap catches, a telephone answering service was set up by ADAS, Cambridge, and Processors and Growers Research Organisation (PGRO). Farmers are encouraged to contact one or other organization after achieving threshold catches of moths, and are provided with a computer prediction of the first effective spray date(s) for their crop(s). In addition, specialist advice is available to deal with particular problems; Rothamsted staff maintain contact with both advisory organizations during the season to provide guidance on trap catch interpretation.

During 1981–82 a joint experiment (RES/ADAS/PGRO) was done in vining peas at 34 sites in four countries to develop a pea moth monitoring system suitable for this crop. The purpose of this system will be to determine whether control measures are necessary

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at all rather than when to apply them, which is the principal objective of traps in the dried pea crop. The attractant used was the synthetic natural pheromone *E,E*8,10-12:Ac (200 μg) combined with an antioxidant (*Rothamsted Report for 1981*, Part 1, 132) on a natural-rubber lure. This was mounted in a trap similar to that used commercially in dried pea crops. In each field a single trap, positioned at least 150 m from the nearest headland was orientated with its long axis along the mean wind direction and examined daily. Records of daily moth catches, crop phenology, damage levels in sprayed/unsprayed crops and in viner samples were obtained. During the two years of the experiment, although large numbers of male moths were caught in the traps, very little pea moth damage was recorded (only two sites had more than 1% peas damaged in unsprayed plots). Moths were first caught at most sites within a few days of one another, but there was no clear relationship between crop phenology and the influx of moths into the crop as shown by trap catches. Other work (see above) indicates that the traps used in the experiment may have attracted moths from outside the crop. If so, these traps will not indicate the influx of moths into the crop, but may indicate whether there is a risk of attack in that crop from moths in the surrounding area. (Garthwaite and Wall, with Greenway, Insecticides and Fungicides Department, Mr J. King and Mr A. J. Biddle, PGRO, Mrs J. Bloodsmith, ADAS Cambridge and Mr B. Emmett, ADAS Leeds)

In the 3 years since pheromone monitoring trials for diamond back moth began in this country no significant damage has occurred in the field, so it has not been possible to relate catches to need for, and timing of, control measures. Nevertheless, growers continue to use the traps as a general, but uncalibrated, indicator of the abundance of moths. An extended fieldwork season has been obtained by co-operation with Dr R. Winney of the Department of Agriculture and Fisheries in Hong Kong where there is greater need to control the large populations of the moth present throughout the year. Experiments there and in the UK have shown that catches in the Far East are enhanced by the addition of 1% of (*Z*)-11-hexadecanol to the lure whereas this diminishes catches in the UK. The presence of small amounts of (*E*)-isomers also appears to have less deleterious effect in Hong Kong.

A field trial of mating disruption is being attempted this winter in Hong Kong using a microencapsulated formulation of a pheromone.

Pheromone supplied to Dr J. Theunissen of the Research Institute for Plant Protection, Wageningen, Netherlands, and used in the Philippines, confirmed results from Rothamsted and Hong Kong monitoring traps; in particular, for this species the sticky surface of the standard delta trap appears to be completely covered with moths after about 250 have been caught; thereafter its efficiency is greatly reduced. When sticky traps are used for comparison of attractant chemicals or in monitoring systems it is necessary to ensure that each sticky surface is large enough to prevent saturation by moths within the interval between consecutive examinations. (Macaulay, with Dawson, Liu and Pickett, Insecticides and Fungicides Department)

Insect pathogens

The host cuticle as a barrier to infection by Entomophthorales. Work on the fundamental mechanisms of insect infection by pathogens continues to make steady progress. In nature, species of fungi of the order Entomophthorales often attack only one species of insect or, at most, a restricted range. Work has been under way to determine the extent to which such limited host ranges are caused by inherent resistance in non-hosts or by ecological constraints.

The first element of host resistance encountered by an invading fungus is the cuticle which provides a physical and chemical barrier to infection. Accordingly, the activity of

https://doi.org/10.23637/ERADOC-1-129

conidia of several entomogenous fungal species on the cuticle of susceptible and non-susceptible insects of economic importance was studied in the laboratory.

With *Entomophthora muscae* (strain nos. X023 and X0023) an adhesive pad formed between the conidium and the cuticle of the housefly, *Musca domestica*, a susceptible host. A germ tube then began to penetrate the integument immediately adjacent to the conidium from which it emerged. The cuticle reacted by melanizing locally, and frequently lifted outwards around the germ tube. A highly characteristic tri-radiate crack formed in the cuticle at each penetration point. Possibly fungal extracellular enzymes induced chemical changes within the cuticle, causing it to absorb water, swell and split to provide access for the germ tube. Germ tubes of the aphid pathogen *Conidiobolus obscurus* (X17) behaved similarly both on houseflies and aphids; the cuticle melanized locally, and a crack formed but this was typically tetra- rather than tri-radiate. Germ tubes of another aphid pathogen, *Erynia neoaphidis* (X4) also caused the cuticle of flies (and aphids) to melanize but they formed a round aperture through which the fungus entered, suggesting a more complete digestion of the cuticle than by the other species. Germ tubes of each of the three species continued their development through the cuticle and epidermis of houseflies into the haemocoel. However, only those of *E. muscae* developed further; hyphal bodies multiplied in the haemocoel and eventually killed the host. This suggests either that housefly tissues are an unsuitable medium for the aphid pathogens or that only *E. muscae* can overcome the humoral or cellular defence reactions of the insect.

Fourth instar larvae of the wax moth, *Galleria mellonella*, were challenged with conidia of another strain of *E. muscae* (X27). The cuticle melanized so extensively that it was impossible to observe specific penetration points. The fungus, normally regarded as a pathogen only of adult Diptera, completed development in, and killed some of the larvae. Conidia from these cadavers were infective for houseflies.

These studies show that in sustained conditions ideal for invasion, the cuticle of some insects fails to resist invasion by fungi usually regarded as non-infective for that host. However, in nature where conditions are normally less consistently suitable, the fungus may be inactivated by ultraviolet light or other factors before it has time to invade any except highly susceptible hosts. (Wilding and Brobyn)

Production of *Erynia neoaphidis* for field distribution to control aphids. Infective material of the aphid pathogenic fungus *Erynia neoaphidis* (Entomophthorales) is required to test the effect of treating aphid populations in the field. Although the fungus grows *in vitro*, this cannot, yet, be prepared in a form suitable for field use. Consequently, in preliminary trials the fungus was established in field populations of aphids by releasing living aphids infected in the laboratory. This method of production allowed only an imprecise estimate of the dose applied and limited the area that could be treated, because all the infected aphids comprising the inoculum had to be available for release during a short period; so alternative ways of producing the fungus were sought. The fungus remained alive when the air-dried bodies of pea aphids (*Acyrthosiphon pisum*) killed by the pathogen were triturated to a coarse powder. When moistened, infective conidia were produced. Doses of 0·05, 0·1, and 5·0 mg of the powder sprinkled on 10 aphid-infected wheat seedlings in an area of 60 cm^2 killed means of 34, 49, 67 and 55% respectively of *Sitobion avenae* and 23, 24, 49 and 60% of *Metopolophium dirhodum* after 5 days. Results were similar for *A. pisum* and the bean aphid *Aphis fabae* on broad bean (*Vicia faba*) seedlings.

Application of the powder established infection in field populations of *A. fabae* as effectively as the release of living infected aphids. As an inoculum, the powder has the advantages that (i) a specified dose can be applied and (ii) the material can be stored for at least 2 months without losing infectivity. Large quantities can be accumulated by

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successive weeks of production and it can be applied when conditions are most suitable. (Wilding and Mardell)

Elemental analysis of insects

The assessment of elemental analysis as a method of distinguishing insects originating from different host plants or regions (*Rothamsted Report for 1980*, Part 1, 98) has continued, with encouraging signs of success. Satisfactory techniques ensuring that specimen preparation should not be an important source of error for either wavelength dispersive (WD) or energy dispersive (ED) X-ray analysis have been evolved for several species. Analyses of many individual insects have been evolved for several species. Analyses of many individual insects have been completed, for the moth *Noctua pronuba* by WD analysis, and for the cereal aphids *Sitobion avenae* and *Rhopalosiphon padi*, the rice planthopper *Nilaparvata lugens* and two species of *Culicoides* by ED analysis. Analysis of *Agrotis segetum*, the most important species of cutworm, awaits new instrumentation, and, for unknown reasons, it has not been possible to prepare consistent samples of wheat bulb fly, *Delia coarctata*, and another cereal aphid, *Metopolophium dirhodum*, for ED analysis.

Elemental data are subjected to multivariate analysis, using principal component analysis (PCA) after transformation to logarithms. It has been found useful to re-analyse sub-sets of the data, by excluding some of the samples in particular groups that seem disparate, or investigating sub-sets of elements, to try to identify those that are important.

N. pronuba has been most intensively studied. Moths have been reared on two host plants, lettuce and broad beans, each host plant being grown in four different soils: two clayey soils representing the extremes of pH at Rothamsted (from Sawyers and Whitehorse), a sand from Worksop, Notts, and 'Rothamsted' potting compost, and analysed for 15 elements. PCA of all samples for all elements gave no recognizable groups; elimination of titanium, the element of most erratic occurrence, indicated that samples were segregating according to host plant. The elements fall into two groups; one, designated 'majors', includes those present at $\geqslant 1000$ ppm (Ca, K, Cl, S, P and Mg), the others, 'minors' at < 1000 ppm (Si, Al, Zn, Cu, Ni, Fe, Mn and Cr; Ti would also be included here). PCA of these sub-sets showed that in the major elements all individuals reared on beans/Worksop were easily distinguishable from the rest, which did not segregate further; neither were distinct groups present within the minor element sub-set. When divided further according to host plant PCA of the resultant host plant/element (major or minor) sub-sets showed clear results. With the exception of individuals from lettuce/potting compost there was no satisfactory separation within either of the host plant/minor elements sub-sets. In the lettuce/major element sub-set, individuals from the Worksop sand and from potting compost separated from each other and from the two Rothamsted soils, but these two soils were not distinguishable; on all soils sexes were separable, the element apparently responsible for this being K. The mean K content ($\pm$SE) of males from lettuce was $16\,624 \pm 260$ ppm, of females $12\,775 \pm 232$ ppm ($P < 0{\cdot}001$). Within the beans/major elements sub-set, all individuals from Worksop were very widely separate from the others, because all major elements were present in very much higher concentrations in all individuals from Worksop sand. Among the other three soils the clearest separation was between sexes, again because of differing K concentrations (males $20\,144 \pm 1221$ ppm, females $16\,514 \pm 1110$ ppm, $P < 0{\cdot}05$), but within sexes the majority of individuals from potting compost were distinct from the majority of those from the Rothamsted soils, which could not be separated.

The cereal aphids *S. avenae* and *R. padi* were each reared on three host plants, wheat, barley and oats, grown in potting compost and analysed for 13 elements, as were samples of *S. avenae* from wheat from localities in Nottinghamshire, Norfolk and Cambridgeshire. Elements again divided into two groups, four 'majors' $\geqslant 1\%$ w/w (Mg, P, S and K)

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and nine 'minors' $<1\%$ w/w (Na, Al, Si, Cl, Ca, Mn, Fe, Zn and Cu). PCA of cultured aphids for all 13 elements gave some indication that individuals from oats were distinguishable from those from wheat and barley. When divided into aphid species/element group sub-sets PCA showed that in *R. padi* host plants were clearly distinguished using major elements, less so using minor elements, in both cases individuals from wheat and barley being more similar to each other than to those from oats. With *S. avenae* there was a similar though much poorer separation, the analysis using major elements again being better than that using minor elements. Individuals of *S. avenae* from the three field samples were not separable in either major or minor sub-sets or in various further sub-sets of the minor elements. The inability to discriminate satisfactorily between samples of *S. avenae* contrasts sharply with clear host plant separation in *R. padi*, but parallels the relative uniformity found in allozyme analyses of *S. avenae* and may be correlated with the fact that, at least in Britain, *S. avenae* is almost entirely parthenogenetic.

The rice planthopper *Nilaparvata lugens* is, for practical purposes, monophagous on rice. Samples from two cultures, in London and Cardiff, and from various localities in South East Asia have been analysed for the same elements as for aphids. The same separation into 'majors' and 'minors' is again obvious. PCA showed that, as with the other insects, the major elements were the most important discriminants. Individuals from the two cultures segregated together whereas those from Asian localities separated widely from the culture individuals and from each other. A consistently higher concentration of Zn separated one sample, from Los Banos, Philippines.

Two species of *Culicoides* are maintained, using the same culture medium, at AVRI, Pirbright; samples of both species have been analysed, for the same elements as aphids and rice planthopper, as well as light trap specimens of one species, *C. nubeculosus*. PCA did not distinguish between cultured individuals of the two species, although they could be separated by allozyme analyses, and light trap specimens of *C. nubeculosus* could be distinguished from cultured individuals by their chemoprints.

Some tentative conclusions can be drawn from the results described. More elements do not necessarily provide better discrimination. A few elements, notably Mg, P, S and K, consistently found in the highest concentrations, can provide discrimination as good as, or better than, that obtained by including more elements which have lower concentrations. However, the differences within and between the species studied suggest that it is desirable to analyse for a reasonable number of elements to allow for selection of sub-sets of elements and to identify elements that, although occurring at low concentrations, may vary widely and consistently enough to distinguish samples without further statistical analysis as, for example, Zn in rice planthopper.

The host plant appears to be a significant factor in the elemental composition of adults of *polyphagous* insects. If this conclusion, which is clear with respect to *N. pronuba*, is confirmed for other polyphagous herbivores, it will mean that for such insects chemoprints would not define reliably geographical sources but could differentiate populations, possibly also identifying populations from different hosts. For *monophagous* species soil type, thus locality, appears to be a major determinant of elemental composition. In principle it should therefore be possible to define geographical sources though with what degree of precision is impossible to say at present; it will almost certainly vary from species to species. The fact that different species of *Culicoides* reared in the same medium (equivalent to the same locality) could not be distinguished whereas different populations of one species could be, suggests that at least some insects with aquatic or semi-aquatic larval stages are comparable to monophagous herbivores. If substantiated this would be important since such insects include many species of major veterinary and medical importance. (Bowden, Sherlock and Rhodes, with Turner, Plant Pathology Department, and Digby, Statistics Department)

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Chemical control and behaviour of slugs

Improvements in the chemical control of slugs are being sought through basic studies on the relative toxicities of different chemicals, their modes of action and other characteristics that control their effectiveness in penetrating the target animal.

Laboratory bioassays have been developed which allow comparison between test materials when artificially introduced into the gut lumen in known amounts, and also for measuring the amount of test chemicals ingested voluntarily in an edible carrier. The relative effectiveness of potential molluscicides can also be compared when acting as contact poisons in laboratory bioassays using substrates of dry glass or moist soil.

Results with disparate chemical compounds and with others in closely-related series, indicate that a number of toxic mechanisms are effective against slugs. Many cholinesterase inhibitors and uncouplers of oxidative phosphorylation are lethal; some novel materials of known action were also toxic but none of the pyrethroids tested has shown any activity against slugs. The apparent toxicity of poisons is greatly affected by the method of application as their ability to penetrate to sites of activity depends on their physical properties. For example, methiocarb, the carbamate poison widely used in edible baits for slugs, is moderately toxic when placed in the gut lumen of the slug *Derocerus reticulatum*, but is almost completely ineffective as a contact poison, being insufficiently polar to penetrate the external mucus barrier in quantity.

Carbamates as a group show molluscicidal activity, with oxime carbamates generally more active. Unfortunately, activity appears in most cases to be linked to repellency, which prevents their use in baits and limits their effectiveness as contact poisons. Some progress has been made in masking repellency using novel formulations which may allow the effective field use of such active but otherwise repellent materials identified in the laboratory. (Henderson, with Briggs, Chemical Liaison Unit and Pickett, Insecticides and Fungicides Department)

Slugs are vulnerable to chemical control only when moving or feeding, and since they are only active intermittently, at night and when microclimatic conditions are favourable, information about the factors influencing activity is essential to improve control. Field and laboratory studies on the ecology of slugs in the potato crop have begun using marked individuals to study movement. *D. reticulatum* is best marked by feeding on one of six vital stains, while darker, tougher-skinned *Arion* spp. can be individually freeze-branded with liquid nitrogen-cooled wires. Individuals marked by both these methods remain identifiable for at least eight weeks. In still-air laboratory tests *Arion hortensis* moved towards sources of volatile plant extracts and similar reactions were elicited by synthetic materials such as methional, the aldehyde component of potato volatiles. (Airey and Henderson)

The use of earthworms in waste disposal and protein production

Basic biology and ecology. The main species used in this programme continues to be *Eisenia foetida* (Savigny) and further basic work on its ecology has been completed. However, it has been confirmed that this 'species' is actually two morphologically identical species separable on colour and physiology. Preliminary work has indicated that the striped species (*Eisenia foetida foetida* (Bouché)) is favoured by moister conditions, and can withstand a greater degree of anaerobicity than the uniformly reddish species (*Eisenia foetida andrei* (Bouché)). Work on optimum moisture conditions has shown that although these species are tolerant of moisture contents between 50% and 90% they tend to grow most rapidly between 80% and 90%. The most rapid increase in total biomass occurred with a ratio of worms to animal waste of 1:50. At lower stocking rates down to 1:600, cocoon production was higher but biomass was produced more slowly, and at higher stocking rates reproduction was very limited.

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Other species of worms have been studied. A laboratory comparison between the growth rate of *E. foetida andrei* and *Dendrobaena veneta* (Rosa) in pig waste showed that *D. veneta* grew much faster and individuals attained a much greater mature weight than *E. foetida andrei*, and each cocoon contained usually only one hatchling compared with 2-4 for the latter species. *D. veneta* may grow and reproduce better in field scale trials since it is a much longer worm than *E. foetida andrei* and mates more readily when not restricted for space. (Edwards and Neale)

Another species imported from the Philippines, showed considerable promise, growing much faster than *E. foetida andrei* and producing up to 20 cocoons per week. It can also withstand higher temperatures and grows and reproduces well at 30°C, a temperature unsuitable for *E. foetida andrei*. (Edwards and Williams)

Several other species are being bred and optimum conditions for growth and worm/waste stocking rates are being studied. (Edwards and Jones)

Food value of earthworms and feeding trials. The processing of worms for animal feed was investigated using various drying and ensiling methods. Heat drying and ensiling with 3% formic acid caused no loss of nutrient value and the preserved product was suitable for incorporating in animal feeds. Chicken feeding trials are nearly complete. (Edwards and Lofty, with Dr J. Worgan, National College of Food Technology, Reading)

Productivity of different wastes and large scale systems. The suitability of pig and cattle wastes for breeding *E. foetida andrei* has been confirmed in laboratory and field trials. Further trials with duck waste have shown this to be an excellent medium for worm growing although the turnover time may extend to more than a month due to the high content of straw and shavings. The conversion ratio of waste to worms was about 5%. Potato peelings, by-products of crisp and frozen chip processing, proved to be an acceptable medium for *E. foetida andrei* with a conversion ratio of waste to worms of up to 5%. Work on growing worms in used mushroom compost with the addition of animal wastes is continuing. (Edwards, Lofty and Jones, with Mr J. Richards and Mr B. Wilson, Cherry Valley Farms, Dr M. Kirkman, Walkers Crisps, and Mr J. Delves-Broughton and Dr D. Doling, Rank Hovis McDougall)

Two pilot worm-growing systems were built in 1982, one at Rothamsted and one at Bore Place, Kent. Both incorporate beds of waste heated from beneath by electric cables with thermostatic temperature control, overhead lighting, and fine mist spray watering, housed in a polythene tunnel. In the Rothamsted system, eight beds 5 m × 3 m × 0·5 m deep were used. The base is a steel grid which can support a small front loading tractor to load the waste. Beds are either filled completely or by the frequent addition of 10 cm deep layers, the latter being the more productive method. Once populations of *E. foetida andrei* have built up, successive layers are worked fully in 2–3 days. At Bore Place, equipment for adding metered quantities of animal slurry to the breeding beds was installed. At Cherry Valley Farms where the worms work the waste in uncovered areas, the fluctuations in temperature and moisture gave poor population increases. In all these experiments the importance of a large initial inoculum of worms was apparent. (Edwards, Lofty and Bater, with Mr D. Knight, Open University, Mr S. Crocker, Commonwork Enterprises, and Mr J. Richards, Cherry Valley Farms)

The mechanical separator built from a design developed by the National Institute of Agricultural Engineering (*Rothamsted Report for 1981*, Part 1, 105) was modified by lengthening it to accelerate throughput and fitting hoppers through which worm-worked waste of three particle sizes is loaded into plastic bags.

A detailed economic feasibility study which assessed the profitability of worm production and waste processing using different wastes, production methods and markets for

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the products was made. This showed the greater profitability to be with larger pig and cattle units. A mathematical model to predict suitable worm stocking rates for different wastes and temperatures was developed. (Edwards, with Mr K. Hepherd, Mr N. Foote and Mr R. Fieldson, NIAE)

Utilization of earthworm worked animal wastes. The potential of worm-worked wastes in horticulture was tested extensively. The worm-worked wastes had considerably more available P, K, Ca and Mg than the parent materials. Most of the nitrogen was changed from the ammonium to the nitrate form and, with the exception of duck waste, there was a considerable increase in available N. The pig waste tended to have quantities of nutrients too high for direct use in horticulture and needed dilution for plant growth trials. Moisture holding capacity of worm-worked wastes equalled that of peat. Trials at Rothamsted showed that germination and early growth of peas, lettuce, wheat and radishes were better in worm-worked cattle wastes than in recommended potting compost but establishment was poor for tomatoes. Work at NVRS showed that lettuce grown in cattle wastes and then transplanted produced yields equal to those in recommended potting compost. Chrysanthemums and antirrhinums grown at GCRI in undiluted wastes with the addition of nutrients did less well than in peat with the same nutrients. The worm-worked waste made a good mushroom compost. (Edwards and Bater, with Johnson and Cosimini, Soils and Plant Nutrition Department, Dr E. Cox, NVRS, Mr A. C. Bunt and Mr P. Flegg, GCRI)

Earthworm/microorganism interactions. The pilot work reported in 1981, (*Rothamsted Report for 1981*, Part 1, 104) on changes in bacterial and protozoan populations in worm-worked animal wastes, was extended by the appointment of an ARC post-graduate student and a temporary HSO with special MAFF funds. Initially, microbial populations in worked cattle and pig wastes are being studied with the aim of identifying major food sources for *E. foetida andrei*. Most organisms isolated to date have been Gram-negative bacilli of the general *Acinetobacter*, *Citrobacter*, *Enterobacter* and *Serratia*, holotrichid ciliate protozoa, e.g. *Tetrahymena*, and Fungi Imperfecti, e.g. *Aspergillus fusarium* and *Trichoderma* sp. The role of the different microorganisms is being assessed using radioactive labelling techniques. Preliminary investigations into rearing axenic *E. foetida* for nutrition studies have concentrated on the cocoons. The internal fluid of the cocoon contains bacteria of the genera *Nocardia*, *Alcaligenes* and *Pseudomonas*. The possibility of the internal cocoon fluid containing a bacteriostatic substance that might retard bacterial development is being investigated. (Morgan and Burrows)

Testing the toxicity of industrial chemicals to earthworms. Two standardized laboratory test methods were developed in 1981 (*Rothamsted Report for 1981*, Part 1, 105) for use by the EEC and OECD. These used either a contact filter paper method or an artificial soil with *E. foetida andrei* as the test organism. The LC50's reported from 60 collaborating laboratories worldwide using the filter paper method were 0·006 mg cm^{-2} for pentachlorophenol, 0·007 mg cm^{-2} for carbaryl, 0·109 mg cm^{-2} for trichloracetic acid (TCA) and 0·023 mg cm^{-2} for the standard copper sulphate. Corresponding LC50's for the artificial soil were 68, 69, 1056 and 1253 mg kg^{-1} respectively. The contact filter paper test gave the greatest reproducibility with every laboratory rating the relative toxicity correctly but results were difficult to relate to field conditions. The results from the artificial soil test were more variable and involved more time and labour but were closely related to field data, TCA and copper sulphate being largely inactivated in the artificial soil as in a field soil. Various modifications to the test methodology were made and a second ring test involving 24 laboratories began in 1982. In this test the unknown com-

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pounds sent out for further assessment were pentachlorophenol, chlordane, potassium bromide with chloroacetamide as a standard. The same compounds have been tested at Rothamsted using a variety of published test methods including forced feeding, immersion, incorporation of toxicants in boxes of natural soil, and tests in a matrix of fine silica and glass balls. These all gave a similar ranking of toxicity to the Rothamsted methods but were much more laborious and less standardized. The results of the Rothamsted methods are being related to those from two field sites with organic matter contents of 5 and 7%, using the chemicals tested in the ring test. The degree of correlation so far has been good. (Goats and Edwards)

Staff

Links with Europe were maintained through Staff attending various Society, IOBC/WPRS, EPPO/WMO and INRA meetings. H. Loxdale was supported by the Royal Society to collect cereal aphids in Spain. More distant visits included one by C. Wall to the University of Massachusetts, USDA, Gainesville and the Southern Grain Research Laboratory, Tifton, Georgia, to discuss pheromone research, by R. Bardner on an FAO consultancy to Uganda and Malawi, by C. A. Edwards to the 2nd Philippines Earthworm Conference in Manila and a UNESCO consultancy to Ghana, and by J. B. Free to the Sultanate of Oman and Kenya to advise on research programmes and initiate experiments with pheromone lures.

N. Wilding, with help from Patricia Brobyn, Susan Mardell, Brenda Ball and P. L. Sherlock, organized an 'Entomophthorales Foray' as part of the 3rd International Colloquium on Invertebrate Pathology, and Departmental members attended and spoke at a Cereal Aphid meeting at University of Southampton and at the British Crop Protection Council Symposium on 'Decision making in the practice of crop protection'. C. A. Edwards presented papers to symposia/colloquia on 'British Agriculture' and the 'Upgrading of Food Wastes' and lectured on this subject to the British Society of Soil Science and to farmers groups; with Departmental staff he contributed an exhibit on 'Charles Darwin and Earthworms' at the Royal Society's Soirées. J. B. Free lectured at the Universities of Cambridge and Reading, and completed his term of office as Chairman (British Section) of the International Union for the Study of Social Insects. T. Lewis lectured at the University of Nottingham, Fisons Boots Limited and Monks Wood Experimental Station on aspects of the Department's work.

L. Bailey retired after a distinguished research career in insect pathology. P. Ives, P. Townley, Anita Elleray, Barbara Goult and J. Doran resigned; G. A. Bent, Jacqueline Fountain, R. Harrington, I. Burrows, Nicola Rowe, M. Russell and Katherine Wilkinson were appointed, and Maxine Morgan took up an ARC studentship. We were pleased to welcome many visiting workers, including Dr R. Krejzova, Cheng Xia-nian, Rini Scholten, Dr Aida Hakin, Dr El Garhy, Dr M. Dogaroglu, Miss Chutamas Satasook and three bee workers from Burma; sandwich students Fiona Ross, Jacqueline Rhodes and T. Williams, and voluntary workers Margo Harris, Lucy Howes and J. Biggin. C. A. Edwards received Individual Merit Promotion to SPSO; C. Wall and Brenda Ball were promoted, and J. B. Free was awarded an Sc.D. (Cambridge).

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