ROTHAMSTED RESEARCH

Rothamsted Experimental Station Report for 1985

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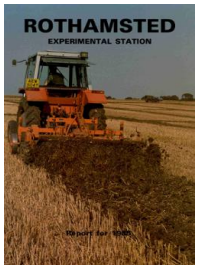

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INTRODUCTION

With the financial cuts indicated in the last annual report now implemented and others foreshadowed, the year has been one of restructuring to make the most of declining resources. Despite this unfortunate situation, remaining staff have responded positively. There is much evidence that the Crop Protection Division at Rothamsted is highly regarded by outside bodies, both public and private, who wish to see a continuation of our work on a wide range of important research programmes. The evidence for this comes from the many

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grants attracted from diverse funding bodies. In the past year awards and contracts exceeding £1 million extending over the next three years have been won. Of course, the Division will only continue to attract funds on this impressive scale if it can maintain a core of high quality permanent staff with confidence in their future.

As well as further support from the British Technology Group for work on the highly successful structure/activity relationships of insecticides, three major agrochemical companies have provided funds for work on the important topics of resistance, pest monitoring and biological control, reflecting the mounting concern with environmental issues. Three small grants related to such problems have also been received from the EEC and the Nature Conservancy Council. Three larger grants have been offered by the Overseas Development Administration covering studies of cereal viruses in developing countries, prokaryote pathogens of plant vascular systems, and the biochemical identification of root knot nematodes. These studies are in accordance with the AFRC's recent 'Forward Policy' which encourages more support for Third World agriculture. It is pertinent to note that there are many staff at Rothamsted with overseas experience able to capitalize on this policy change. Two less obvious sources of funds are the US Army which supports work on earthworms and arthropods as indicators of heavy metal residues in dredged soils, and the Health and Safety Executive which is funding studies on the detection of airborne allergens. The Division has so far managed to attract a reasonable share of MAFF competitive funds, two AFRC New Initiatives, and established links with many universities through SERC/CASE awards and AFRC-linked studentships.

In a situation where adaptability and versatility are the key to progress this response to new financial and scientific challenges has been most gratifying. At the same time, Divisional staff have continued to provide a favourable image to the farming community through their presence at many demonstrations, exhibitions, agricultural shows and meetings throughout the country.

ENTOMOLOGY DEPARTMENT

As staff members decrease, work on a wide range of topics related to aphids, their insect enemies and pathogens, and their virus vectoring capacity, is emerging as a dominant theme of the Department's activities. Of course, other groups of insects still pose serious pest problems and present opportunities for good scientific research, but inevitably there has to be a narrowing of interests in present circumstances. Excellent environmentally orientated work on moth monitoring and midge biology continues, and the Department retains unique expertise on honeybee management and diseases, but the input into studies on soil arthropods and earthworms has been severely reduced after more than forty years of effort that has contributed much to the Department's international reputation. This report concentrates on aphid-related studies and such work on honeybees and soil invertebrates as has been possible.

Aphid studies

Surveying. The national network of suction traps continued to operate and an additional trap was sited at the Leeds University farm specifically to study the barley yellow dwarf virus problem in that part of England. The pest aphid information obtained from the traps continued to be circulated by mail to interested parties within the agricultural industry in the form of the *Aphid Bulletin* and *Aphid Commentary*.

The use of computers on farms has increased greatly during the last few years, and recently specialist viewdata systems for the agricultural industry have been introduced. During 1985 aphid information was made available to the agricultural industry on Prestel Farmlink, the

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first of these agricultural viewdata systems, thus providing the industry with access to the information more rapidly. The information on Farmlink was updated weekly from May to August and included the aphid pests of cereals, potatoes, sugar beet, field beans and hops. Occasionally information for peas and carrots was also given as *Acyrthosiphon pisum* and *Cavariella aegopodii* occurred in unusually large numbers during 1985.

The information on Farmlink described briefly the origin of the data from the suction trap network, and gave regionalized reports for each crop. The reports for cereal aphids were accessed most frequently, followed by those for field beans. ICI's Agviser system is likely to provide another outlet by 1986.

The study of factors influencing the field to field variation in cereal aphid numbers within a region, the Rothamsted Insect Survey Cereal Aphid Monitoring Scheme (RISCAMS), and the interpretation of suction trap samples, continued. Sampling was extended to include winter wheat fields near four suction traps in south-east England. As in 1984 (*Rothamsted Report for 1984,* 92–93) weekly summer samples assessed aphid abundance and natural enemy activity in from 10 to 12 fields around each suction trap site.

The winter of 1984/85 was very cold so few aphids survived on crops near Rothamsted; crop development was also retarded as in 1979. The cool, wet June further delayed crop development so that early-sown crops did not flower until the second half of the month. Aphid numbers remained low throughout June, as expected from the few immigrant aphids monitored by the Insect Survey suction traps. Numbers of immigrant grain aphid, *Sitobion avenae,* and rose grain aphid, *Metopolophium dirhodum,* increased at the beginning of July but by then the majority of crops were beyond the most susceptible stages, although some fields were sprayed with an aphicide. The yield losses caused by such late infestations of both species are poorly understood but in a field trial at Rothamsted a 0·4 t ha^{-1} benefit was obtained from a spray at flowering.

Differences in aphid populations between fields within a region were largely due to variations in sowing date, and variety (Rapier again showing partial resistance to *S. avenae* but not to *M. dirhodum, Rothamsted Report for 1984,* 92).

Aphid population development in 1985 was simulated by a mathematical model. The results overestimated early population development, indicating that field survival of immigrants and their offspring was lower than that used in the model, probably due to the adverse weather in June. Omission of these early immigrants improved the fit of the model although modelled population development still occurred earlier than observed. (Tatchell, Carter, Woiwod; Dupuch, French, Mitchell, S. J. Parker, Taylor and D. K. Riley)

Radar detection. The requirement to monitor airborne insect density at various heights in the atmosphere and to provide up-to-the-minute information on such movements for insects of agricultural importance has led to the development of a fully automatic, computer-controlled, ground-based remote sensing system which uses radar to detect and identify individual insect targets.

The radar comprises a high power, short pulse length transmitter operating at a wavelength of 3·2 cm (9·4 G Hz), coupled alternately to one of two parabolic antenna systems. The antenna assemblies are driven by a computer to produce vertically-directed, conically-scanned beams within which the electric polarization of the transmitted pulse can be rotated.

As insects fly through the beams at heights between 12·5 and 250 m, the return echoes are detected and processed in real time using a high speed, multi-processor computer. From measurements of the amplitude and phase of the return echo, the computer calculates the target insects' velocity, height of flight, body weight, shape and orientation. These data are then stored for subsequent transmission over the telephone to a remote central computer.

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The radar system is housed in a purpose-built, all-weather, air-conditioned trailer and is undergoing field trials at Rothamsted.

Ultimately the success of the system will depend upon the degree to which the target signatures can be classified as specific to insect types. This is a new subject area which we have termed 'Radar Taxonomy', and for which New Initiative funding has been granted. The key to identification will be the accurate measurement of the microwave scattering properties of the various insect species likely to be detected by the radar. Preliminary laboratory measurements have already been made on a number of aphid species, the results agreeing with theoretical predictions. These results have been incorporated into a computer model of the Rothamsted radar and agree in detail with actual radar measurements made in the field. Current research is aimed at improving the sensitivity of the laboratory technique to measure the microwave scattering properties of insects weighing as little as 0·1 mg. As these investigations will continue alongside a radar system already capable of detecting gross insect characteristics, such as weight and body shape, new findings will be tested rapidly and incorporated into a practical monitoring system.

Over the next few years several fully automatic, computer-controlled insect radars will be operated at selected suction trap sites in the UK. The resulting information on insect numbers and movement will be transferred immediately via telephone, to Rothamsted, where it will be collated with the latest suction trap and field sampling data and used to provide day-to-day pest warnings and longer-term forecasts. (Bent, Cuminetti; Prior)

Vectors of potato viruses. During 1985, aphids were trapped on a vertical net downwind of a plot of potatoes all infected with potato virus Y, then confined overnight on tobacco seedlings to detect what proportion of the catch was viruliferous; infected plants showed symptoms of the virus a few days after infection. As in 1984, *Brachycaudus helichrysi* was the most important vector, accounting for 29% of transmissions; *Phorodon humuli* accounted for 20% and *Myzus cerasi* for 14%. No *M. persicae* were found to be vectors in 1985 but only three were trapped on the net. Four species not previously recorded as vectors (*M. cerasi, Cryptomyzus ribis, Dysaphis* sp. and *C. aegopodii*) were each responsible for at least one transmission. The work is leading towards an assessment of the relative importance of each aphid species as a vector of potato virus Y which will be incorported in a model of virus spread based on routine aphid data from suction traps and crop characteristics to make it field specific. (Harrington with Gibson, Plant Pathology)

Population genetics. Four years' data on the frequencies of allozyme variants at the glutamate-oxaloacetate transaminase (GOT) locus have now been collected from populations of the holocyclic cereal aphid *Sitobion fragariae* infesting *Rubus fruticosus* and *Dactylis glomerata* within 50 km of Rothamsted (*Rothamsted Report for 1983,* 90). Populations as a whole are genetically variable; at least five alleles exist in nine genotypic combinations at the GOT locus; certain populations examined can be differentiated by their allele frequencies, which remain fairly stable temporally, sometimes even during host alternation, with immigration and emigration of alatae apparently having little effect on established population structure. In some populations, overwintering is predominantly in the egg stage following mating of sexuals on the primary wood host (*R. fruticosus*) in autumn. Because of the stability of allele frequencies during host alternation in these populations, the GOT alleles sampled appear to be neutral or, at most, weakly selected.

In 1984, electrophoretic variation was studied in *Myzus persicae* infesting beet, rape and weeds in south-east England. Four main sites were sampled (Sall, Norfolk; Brooms Barn, Suffolk; Coggeshall, Essex; Rothamsted, Hertfordshire). Aphids were cloned on excised potato leaves in a constant environment, then examined for quantitative variation at the esterase-4 (E4) locus, which confers insecticide resistance in this species, and for qualitative

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variation at 13 other enzyme-coding loci. In all populations sampled, the R1 (resistant) genotype occurred at a frequency greater than 60%, the remaining aphids being mostly susceptible (S). Few R2 (highly resistant) aphids were detected (<3%). Except for esterase-1/2, other enzymes were invariant, suggesting that only a small number of *M. persicae* clones exist within the UK and that although sexual reproduction may be a useful overwintering strategy, it plays little part in generating and maintaining genetic variation in this species.

Similar studies are under way on *Rhopalosiphum padi* and *M. dirhodum.* (Loxdale; Brookes and ffrench-Constant)

Aphid parasitoids. Many insect parasitoids attack a range of host species including crop pests and innocuous hosts on wild plants. The effectiveness of parasitoids against pest hosts is often improved when alternate hosts are present near a crop. Parasitoid populations can survive and build up their numbers on these alternate hosts during periods of low pest abundance.

The aphid parasitoid *Aphidius ervi* has a number of hosts including the cereal aphids *S. avenae* and *M. dirhodum,* the pea aphid *A. pisum* and the nettle aphid *Microlophium carnosum.* It has been suggested that *M. carnosum* could act as a useful reservoir host for *A. ervi*, helping to build up its numbers in spring, or that the parasitoid could alternate between *A. pisum* and cereal aphids if lucerne and wheat were grown in close proximity.

The usefulness of alternate hosts, however, depends on the ease with which the parasitoid will switch between them. Previous olfactory and switching trials with a laboratory culture of *A. ervi* maintained on *A. pisum* suggested a reluctance of the parasitoid to attack either cereal or nettle aphids after being reared on pea aphids for several generations. Recently, further switching trials were done using two *A. ervi* cultures, collected and maintained on *M. carnosum* and *A. pisum* respectively. When *A. pisum*-reared parasitoids were switched to *M. carnosum,* reproduction (measured as mummy production) was greatly reduced, but a switch in the opposite direction did not affect mummy production. As a comparison, similar trials were done using *A. rhopalosiphi,* a parasitoid which is restricted to aphids on cereals and grasses. The two cultures used were maintained on *S. avenae* and *M. dirhodum.* Mummy production was high on *M. dirhodum,* whether the parasite had been reared on *M. dirhodum* or *S. avenae.* However, a switch from *M. dirhodum* to *S. avenae* resulted in far less mummy production compared with that of individuals maintained on *S. avenae* for several generations. Reduced mummy production could result from lower attack rates by parasitoids or from increased encapsulation of parasitoid eggs by the hosts. Results of attack rate trials closely paralleled the switching results suggesting that reduced host recognition followed some host switches.

Reluctance to switch hosts and concomitant reductions in reproduction would limit the usefulness of alternate hosts in pest management systems. The trials will be extended to the field, using wild parasitoid populations which have not suffered the effects of genetic drift common in laboratory cultures, to clarify the importance of the problem. (Powell; A. F. Wright)

Work on the oviposition behaviour of parasitoids of cereal aphids continued, using artificial aphids in bioassays (*Rothamsted Report for 1984,* 95). Aphid models treated with honeydew elicited obvious feeding responses but parasitoids did not attempt to oviposit in them. Distinct oviposition behaviour was stimulated by models treated with an aqueous extract of wheat leaves, the food plant of the host aphids. Control models treated only with water did not elicit a response. The behaviour of the parasitoids suggested that they were responding to volatile components of the plant extract. Oviposition attempts were curtailed if the parasitoid touched the model with its antennae, suggesting that contact kairomones from the host aphid were usually necessary to sustain the behaviour.

Aphid parasitoids appear to respond to a series of olfactory cues, including kairomones

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from the host and synomones from its food-plant. These responses probably play a part in determining the host range and host specificity of individual parasitoid species. (Decker and Powell)

Entomogenous fungi for aphid control. Two lines of work designed to assess the potential of *Erynia neoaphidis* for aphid control, namely production of the fungus and its application in the field, have continued.

Production. Although the fungus grows readily *in vitro* it has not been formulated previously in a way suitable for field use, so for field experiments it has been applied hitherto by the distribution of the triturated bodies of air-dried, fungus-killed aphids (*Rothamsted Report for 1982,* Part 1, 99). In the host, the fungus grows as short hyphal elements, the hyphal bodies, at the expense of the aphid tissues. When the nutrients are exhausted sporulation occurs (conidiophores form at the host cuticle and the production and discharge of the infective conidia commence). On suitable agar and non-agitated liquid media the fungus extends radially by hyphal growth from the inoculum and sporulation occurs in the older part of the culture behind the growing edge. In agitated liquid media however, hyphal bodies develop and multiply but conidiophores do not form. If these hyphal bodies are filtered off and kept moist, sporulation begins after a few hours.

Media based on yeast extract and glucose supplemented with egg yolk or milk support active growth. Recent work suggests that much of the growth promoted by these ill-defined organic nutrients can be achieved by replacing them with 0·1% oleic acid. Growth in agitated liquid medium inoculated with plugs of the fungus from plate cultures on agar media may take 12–14 days at 20°C to reach the stationary growth phase. However, if a suitable liquid medium is inoculated with hyphae from another agitated liquid culture in the log phase of growth, development is much quicker. Growth is optimum when large inocula comprising 40% of the total volume are transferred to fresh medium every two to three days.

Further work is in progress to develop techniques, based on agitated liquid culture, to produce hyphal bodies in large quantities using industrial fermenters, and to formulate the material produced so that it can be stored without losing activity before field use. Also, numerous isolates from many aphid hosts and several regions of the world are being screened for their infectivity, rate of development in aphids and ease of growth *in vitro*. (Wilding; Brobyn and Mardell with Mr P. Robinson, King's College, London and Dr G. Latteur, Station de Zoologie Apliquée de L'Etat, Gembloux, Belgium)

Field application. An experiment in 1985 on field beans, *Vicia faba.* infested with *Aphis fabae* was designed to compare the effect of a mid-June treatment with *Erynia neoaphidis* produced *in vivo* with that produced *in vitro*. Because of culturing problems, the *in vitro* material was not applied until 14 July. Hyphal bodies in suspension produced in a 2 litre fermenter in Belgium were applied in the evening by compression sprayer, less than two days after they were harvested from culture. A laboratory assay conducted on a sample of this material shortly before application confirmed a high infectivity for aphids comparable with that of the fungus growing on the natural host. Further, conidia were discharged from 47% of a sample of leaves collected from the treated plots on the day after the application, demonstrating that some of the fungus material, at least, had survived overnight. Because the application had been made so late the host population in all treatments already carried a 'background' level of infection with *E. neoaphidis* from natural sources, obscuring the effect of the treatment. Nevertheless there was some evidence that the application increased infection in the field which encourages further field testing of the *in vitro*-produced material.

Two applications of fungus-killed aphids to other plots in the same experiment, a treatment which in previous years has consistently and significantly advanced the time when the

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aphid population collapsed, gave a similar result this year. However, as before, the spread of the fungus was too slow to protect the crop adequately from damage. The mean mortality caused by the fungus one week after the first application was only 8%. It is essential that this initial level be increased if control provided by the fungus is to be improved significantly. The fungus requires saturated conditions over many hours to produce conidia and cause infection. In the field such conditions usually persist at the leaf surface during the night but are lost soon after sunrise. Ways of reducing the period from application to infection by applying the fungus material at a later stage in its development and of prolonging the period of high humidity should help to increase the initial infection level. (Wilding; Brobyn and Mardell, with Dr G. Latteur, Station de Zoologie Apliquée de L'Etat, Gembloux, Belgium)

Initial infection might also be encouraged by increasing the activity of the aphids and so increasing the dose of conidia they receive. In a laboratory experiment, young Chinese cabbage plants infested with *M. persicae* were subjected to a shower of conidia of *E. neoaphidis* from infected aphids. Immediately after the application and at intervals of 2 h thereafter, the aphids were excited by treatment with the aphid alarm pheromone, (*E*)-β-farnesene. Three treatments with the pheromone led to a threefold increase in the proportion of aphids infected (from 5·6% to 17·0%) over those not treated. (Mardell and Wilding, with Woodcock, Insecticides and Fungicides)

Honeybees

***Varroa* and virus incidence in adult bees.** The loss of many honeybee colonies throughout the continent of Europe has been attributed to their infestation with the parasitic mite *Varroa jacobsoni*. Previous work suggests that death of infested colonies may be associated with acute paralysis virus (APV) and possibly other honeybee virus diseases. Research in collaboration with European colleagues was undertaken to determine the incidence and prevalence of virus infections in colonies.

Dead bees were collected from beneath the entrances to 18 *Varroa*-infested colonies in the Black Forest area of Germany and 30 uninfested colonies around Hertfordshire. German colonies were divided into three groups (low, medium and high), according to their level of infestation with *Varroa*. Extracts of the dead bees were tested for the presence of viruses by immunodiffusion.

The incidence of black queen-cell virus, bee virus Y and bee virus X was similar in all the colonies sampled and did not appear to be correlated with the level of *Varroa* infestation. All three viruses are only infective for adult bees by ingestion and would not be expected to be transmitted by the feeding activities of mites.

Chronic paralysis virus (CPV) is the only virus in Britain that is both infective for adult bees by injection into the haemolymph and causes natural mortality in the field. In comparison with the British colonies the percentage of German samples with a CPV was much higher, even in the colonies with a low mite population.

Acute paralysis virus (APV) has never been detected by immunodiffusion in extracts of dead adult bees collected from colonies in Britain. By contrast some of the samples from the German colonies in each category of infestation contained sufficient APV to be detected directly by serology. In the low-infested group the incidence of APV was less than 3% of all samples analysed, but in the medium-infested group this had risen to 40%, whilst in the high-infested colonies over 80% of the samples contained much APV. The high incidence of APV in *Varroa*-infested German colonies from July onwards coincides with the steep decline of the adult bee population. This strongly suggests that APV is the primary cause of adult bee mortality in German honeybee colonies severely infested with *Varroa jacobsoni*. (Ball; Allen)

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Detection of acute paralysis virus in *Varroa*. In laboratory experiments individual field-collected *Varroa* mites were shown to be capable of transmitting acute paralysis virus (APV) to healthy honeybee pupae (*Rothamsted Report for 1984*, 99). An enzyme-linked immunosorbent assay (ELISA) was used to determine the suitability of the technique for monitoring APV in mite populations in infested colonies in Germany.

The assay proved to be very specific and sensitive, detecting virus at concentrations as low as 3 ng per mite. Mites collected from both live adult bees and brood contained APV but virus concentrations varied widely between individuals. As much as 1 μg of virus was detected in some mites which had been feeding on APV-infected pupae. Mites ingesting large amounts of APV in this way could transmit virus to their next host. Such transmission of virus by large numbers of mites may cause the unusually large losses of adult bees found in colonies heavily infested with *Varroa*. (Allen and Ball with White, Plant Pathology and Antoniw, Biochemistry)

Alarm pheromones and defence. The collective defence of a honeybee colony against intruders is coordinated by pheromones released from the sting and mandibular glands of alerted or stinging workers. The response to alarm pheromones at the hive entrance has at least five elements: the pheromones alert other workers; they mark the enemy and encourage defenders to sting it; they inhibit foraging so that defenders are retained at the nest; they discourage Nasonov scent release which would give an inappropriate signal; and they repel clustering bees (which may be intruders). Bioassays have been devised to test the effect of individual components of the alarm pheromones on each of these responses. Twenty-seven compounds from the sting apparatus and one from the mandibular glands have been identified. Only isopentyl acetate the most abundant component in the sting was strongly active in all five bioassays. Five compounds (including 2-heptanone from the mandibular gland) were strongly active in four of the bioassays, 12 compounds were active in one, two or three bioassays and only nine compounds failed to show any marked activity or were inactive. Each response was affected by at least seven compounds. The complexity of alarm pheromone action reflects the complexity of the responses required. As different combinations of components elicit different responses, it is possible that honeybees could selectively release components to give different signals according to the behavioural context.

Pheromonal transfer from queens. Pheromones from the honeybee queen are continuously distributed around the colony by antennal contact and have an essential role in the regulation and coordination of colony activity. Worker honeybees obtain the pheromone when they join the court around the queen and palpate and lick her.

Observations have shown that one-year-old mated laying queens receive more attention from workers than newly mated queens which in turn receive more attention than virgin and immature queens; in winter, mated queens are less active and their courts are smaller than in summer. These differences probably reflect the differences in the amount and quality of queen pheromone produced.

Close-up video recordings have enabled a detailed analysis of behaviour in the queen's court. Workers palpate the whole body of a mated laying queen with their antennae but they rarely lick anywhere but the dorsal surface of her abdomen. Workers licking the queen spend longer in the court and usually direct their antennae towards the inter-segmental regions of the abdomen which correspond to the positions of the tergite glands. It seems likely that these glands are the source of an attractive pheromone which may either have a direct role in the regulation of colony activity, or may facilitate distribution of another pheromone such as that from the queen's mandibular glands.

Control of queen production and foraging. When a queen dies or is lost from a colony the reduction in supply of queen pheromone stimulates workers to rear larvae as replacement

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queens. The immature queens themselves provide an inhibitory feed-back signal, probably a pheromone, which prevents the rearing of too many potential queens. Virgin queens have now been shown to be as effective as mated laying queens at inhibiting the rearing of more queens. However, virgin queens are less effective than mated laying queens at inhibiting the production of queen cell cups (the empty precursors of the cells in which queens are reared) which again suggests that there may be qualitative or quantitative differences in the pheromone from mated and virgin queens.

Virgin queens have been shown to stimulate foraging strongly, probably by a pheromone. Although they collected less pollen, colonies with virgin queens foraged as much as those with mated laying queens. Until queen pheromones are identified and synthesized, virgin queens could be used by beekeepers as an additional source of queen pheromone to encourage foraging and inhibit the rearing of queens. (Free; Ferguson, Hadley, Simpkins and Tomkins)

Soil invertebrates

Slug control. Encouraging progress has been made with work to develop effective methods of chemical control without undesirable ecological side-effects. Several types of naturally-occurring chemicals which reduce grain hollowing of wheat seeds, in which the plant 'germ' or embryo is destroyed preventing germination, have been identified. Two such compounds, one of plant origin and the other from an animal source, have shown useful antifeedant activity against the main slug pest of cereals, the grey field slug, *Deroceras reticulatum,* in laboratory tests. They are being evaluated further as seed treatments on winter wheat in an autumn-sown field experiment.

Previous studies on the physical and chemical requirements of slug contact-action poisons have been complemented by studies on methods of enhancing uptake and increasing persistence on wet soil. A number of organic complexes have now been synthesized which show short term (less than a week) activity as soil surface dressings. In a field trial with direct-drilled winter wheat a degree of control was achieved but it was less than that given by conventional poison bait pellets because of the comparatively short persistence of the treatment.

A novel stomach-acting slug poison discovered by laboratory bioassay has also been tested under field conditions. In the single trial made to date it was as effective as current commercial baits and may offer advantages in terms of cost and lower toxicity to birds and mammals. (Henderson; Fisher and K. A. Parker, with Briggs, Pickett, Smith and Woodcock, Insecticides and Fungicides, McGrath, Soils and Plant Nutrition, and Dr J. I. Bullock, University of Surrey)

Exploitation of farm wastes. The Department's programme on using earthworms to process organic wastes from livestock and industrial processes has been curtailed seriously due to withdrawal of MAFF funds, but a commercial company, British Earthworm Technology Limited (BET), based at St Ives, Cambridgeshire, has been formed, and restricted work maintained in collaboration with this firm.

Emphasis has continued to be placed on compost studies rather than on worm protein as animal feed. Further advances have been made by the National Institute of Agricultural Engineering (NIAE) in the engineering and design of bed-loading machinery and continuous processing systems, particularly for the breakdown of straw-based wastes such as farmyard manure. A full-scale, semi-automated wormery (550 m^2 of worm bed) has been erected at Diss using straw-based wastes and incorporating this novel machinery. The bed is being monitored for changes in work populations, rate of waste processing and the economic viability of processing on a large farm site.

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A smaller pilot plant is operating on a pig farm using separated pig solids, and a similar bed has been erected at Biggleswade sewage works to process sewage cake. This project runs in parallel with collaborative work between BET and the Water Research Centre (WRC), Medmenham, to identify suitable sources of sewage cake for treatment with worms and optimum environmental conditions in which to carry out the process.

Work on the population dynamics of *Eisenia foetida, Eudrilus eugeniae* and *Perionyx excavatus* has continued with a view to increasing rates of waste breakdown in continuous processing systems. (Edwards, Bardner, Fletcher, Lofty and Neale, with Dr J. R. Phillips, NIAE, Dr J. A. J. Mullett, BET and Mr J. Hall, WRC)

Plant growth trials. The potential of worm-worked wastes as components of horticultural growing media continued to be investigated with help from Oaklands Agricultural College. At Rothamsted, a range of compost blends suitable for commercial use was developed, consisting of general purpose growing media, soil conditioners and composts for species with specific requirements. Test plants were generally as good as, or better than, those grown in commercially available composts, with early seedling emergence and early flowering noted in certain plants, particularly calceolaria.

Straw-based worm-worked wastes were further investigated for their potential use in compost blends. Residual straw was sufficiently decomposed to prevent any plant retardation due to nitrogen immobilization, enabling them to be used in commercial growing media. (Burrows; Cornwell)

Earthworm and microbial interactions. Work on the nutritional requirements of *Eisenia foetida* ended in June 1985, and though they were not defined precisely, it was shown that *E. foetida* required a variety of microorganisms and products of microbial decomposition to grow and develop.

E. foetida was cultured in cattle, pig, horse and poultry manures, and the microbial population of these was characterized and enumerated. Results showed a good correlation between worm growth and number of microorganisms, indicating that they are important factors in the worm diet.

The nutritional value of various microorganisms common to a variety of wastes was tested using cultures of worms maintained in sterile, inert environments. The worms were treated with antibiotics to render them both internally and externally free of microorganisms before being fed on pure cultures of bacteria, fungi or protozoa. Fungi were shown to form a substantial part of the diet and a range of species of bacteria, particularly pigmented colonies, were toxic to worms.

Proteins, carbohydrates and lipids were also tested as possible food sources, both in cattle waste and in synthetic media. Neither proteins nor lipids were satisfactory as food but carbohydrate (glucose at 1% w/w in a cattle waste medium) was beneficial and increased worm reproduction. (Morgan, Edwards and Burrows, with Dr J. Beringer, University of Bristol)

Earthworms and heavy metal residues. Research sponsored by the US Government has continued to assess the suitability of an earthworm bioassay to indicate the bioavailability of heavy metals present in soils and sediments. Once validated, such a bioassay may be applied to sediments and sludges dredged from waterways and harbours to assist in making decisions on management of areas onto which the material has been disposed.

The bioassay was developed at the Waterways Experiment Station (WES), USA, using the earthworm *E. foetida*. This species exploits substrates containing high levels of organic matter, such as manures and composts and is unlikely to be found in habitats created at dredged material disposal sites.

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At Rothamsted, verification of the earthworm bioassay procedure has been approached in laboratory and field. Laboratory studies have compared the uptake of heavy metals by *E. foetida* with uptake of metals by five other species of earthworms commonly found in soil/sediment substrates. Ten soils/sediments were selected for assessing the bioassay. These included mining contaminated soils from Wales and Somerset, and sediments from polders and rivers in Holland and Germany containing a range of concentrations of the elements Zn, Pb, Cu and Cd. Uptake of heavy metals has been measured in at least four earthworm species including *E. foetida* grown in each of the 10 soils/sediments over periods of 15, 28 and 56 days. Some modification of the WES bioassay procedure was necessary for application to the field species of earthworm and to ensure valid comparisons between species.

In the field, at existing dredged material disposal sites in the USA, earthworms and other soil-dwelling invertebrates naturally colonizing the sites have been collected and analysed for heavy metal content. These results are being compared with results of heavy metal uptake measured in *E. foetida* grown under laboratory bioassay conditions using dredged materials taken from these disposal sites.

Although in many respects earthworms represent ideal indicator organisms for monitoring the bioavailablity of heavy metals present in soils, sediments and sludges, a major drawback has been distinguishing between the heavy metal burden due to uptake into the earthworm tissue and that resulting from soil within the earthworm gut. A method has been developed which utilizes acid insoluble residue as an inert marker to enable the quantity of soil present in any earthworm sample to be calculated. A correction factor can then be applied which eliminates the heavy metal concentration resulting from soil within the earthworm gut leaving only the concentration of heavy metals incorporated in the earthworm tissue. (Stafford, with Cosimini and McGrath, Soils and Plant Nutrition)

Staff and visiting workers

During the year C. A. Edwards retired, and the posts of six staff, R. Bardner, J. R. Lofty, Joan Nicklen, J. E. Bater, Barbara Jones and Dolores Sturgeon, were declared redundant. It is a pleasure to acknowledge their varied contributions to the studies and life of the Department over many years.

Sixteen staff participated in a wide range of international and UK Society meetings and working visits. These included studies on biological control in the Peoples' Republic of China by W. Powell, and on pest monitoring in Hungary by G. M. Tatchell and I. P. Woiwod. E. D. M. Macaulay tested propheromones for cotton pest control in Pakistan, N. Wilding attended a conference on Diamondback moth management in Taiwan, C. Wall attended the 2nd Annual Meeting of the International Society of Chemical Ecology in Wisconsin and gave lectures in Denmark and Sweden, J. B. Free attended the 30th International Apicultural Congress in Japan and visited Bangladesh, and C. A. Edwards gave a lecture tour in Finland and attended sugar beet and IOBC integrated control meetings in Brussels and Wageningen along with W. Powell, J. R. Lofty, J. E. Bater and Barbara Jones. C. A. Edwards and K. E. Fletcher were present at an Earthworm Symposium in Bologna, Italy; G. M. Tatchell, I. P. Woiwod, R. Harrington and M. S. Taylor held discussions with aphid surveying colleagues in Montpellier, and H. D. Loxdale, R. Harrington and T. Lewis visited Smolenice, Czechoslovakia for an international symposium on aphids and thrips. Many staff attended and spoke at various symposia in the UK organized by the Association of Applied Biologists and the Royal Entomological Society.

Linda Muir and N. Morris resigned. Caroline Mitchell, S. Prior and R. Cuminetti were appointed, the latter transferring from the Department of Physiology and Environmental Physics. Nine students or visiting workers joined the Department for varying periods—

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Professor S. Takeda from the University of Gifu, Japan, M. S. Clough, Kathryn J. Dancy, B. Farrell, A. Hartley, Karen Healey, Alison Howe, D. Morgan and Ute Seibt. W. Powell, H. D. Loxdale and Jacqueline R. Simpkins were promoted. Maxine Morgan completed her Ph.D. studies and I. Burrows, M. Cornwell and Elspeth Neale remained as visiting staff supported by British Earthworm Technology Limited.

T. Lewis was elected President of the Royal Entomological Society for a two-year period.

INSECTICIDES AND FUNGICIDES DEPARTMENT

As noted in the Divisional introduction, the Department continues to obtain outside funding for work with obvious and immediate agricultural implications. However, the main thrust of the Department's effort is basic research into improving chemical crop protection and minimizing its environmental impact. The product of this research must be developed by industry for eventual use by farmers and close links with agrochemical firms have been established so that their views can be considered at the research planning stage. Substantial encouragement has been received for the general programme, exemplified by wider testing by industry of our new insecticides and behaviour-controlling chemicals, and in the advice sought from staff for dealing with the rapidly developing problem of insecticide resistance.

Further cuts in funding have limited the research programme to five main topics and the Chemical Liaison Unit has been disbanded. The only remaining work on fungicides is in the physicochemical parameters group, now led by R. H. Bromilow, with general implications for fungicide development and in particular for the design of compounds active against take-all. Studies on new spraying technology designed to improve use of established crop protection agents and to minimize their environmental impact have ceased, and the work of the remaining staff has been channelled, under the leadership of D. C. Griffiths, into the design of systems for deploying novel crop protection agents such as new types of insecticides and the behaviour-controlling chemicals being developed in the Department. In the future this group will attempt to improve application of biological agents and of translocatable compounds provided by the physicochemical parameters group.

In spite of financial stringency, the AFRC has provided the Department with a new mass spectrometer and a new high-field NMR. The mass spectrometer (70–250 VG Analytical) is a much more sensitive instrument than was previously available and will be used mostly for the identification and development of new behaviour-controlling chemicals. The NMR machine (GX400 JEOL) has a high-field magnet of 400 MHz and will allow more effective characterization of synthetic intermediates and insecticides and also of other putative crop protection agents.

Relationships between molecular structure and insecticidal activity

The need to discover new pesticides with environmentally favourable properties, and with the ability to control populations of insects resistant to established insecticides, is urgent. Basic studies, such as those reported last year (*Rothamsted Report for 1984,* 102) can contribute by identifying novel structures, and may lead to compounds worthy of development. Potent insecticidal activity, and consequently reduced environmental loading, remains a primary target. In addition, novel structures imply the possibility of altered responses to resistance mechanisms, so the performance of the new compounds against resistant strains of insect has been examined.

Synthetic pyrethroids with a non-ester central group. Further structure-activity relationships have been established in the series of compounds represented by structure (I), for which preliminary results were reported last year. Conclusions are as follows:

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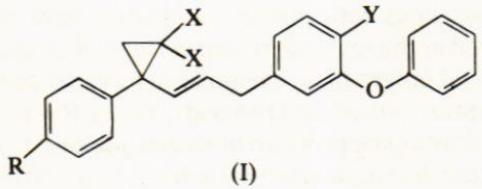
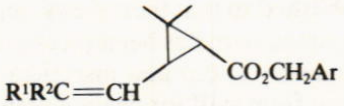
1. Introducing fluorine into the cyclopropane ring (changing X from H to F) produces only small changes in insecticidal activity, for the compounds examined.
2. The enhancement in activity on changing Y from H to F (noted last year) is remarkably consistent for a series of R groups, of forms of the gemdimethyl group (cyclopropyl in (I)) and of the link ($-CH=CHCH_2$ in (I)). For houseflies, the enhancement is 1·73 (0·37–5·82) and for mustard beetles 4·32 (1·57–8·15) for the 17 pairs of compounds examined.
3. Other forms of the alcohol moiety were examined by replacement of the 3-phenoxyphenyl group in (I). Even those chosen for their known effectiveness in other pyrethroids proved inferior, so an unpredicted interaction between the contributory parts of the molecule must influence the overall activity of these compounds.



(I)



(II)

Enol ether side chains. Replacing the isobutenyl (II; $R^1=R^2=Me$) side-chain in chrysanthemates by an enol ether group (R^1 or $R^2=OAlk$) retains insecticidal activity despite inherent lability in acidic media. When $R^1=H$, effectiveness decreases in the order $R^2=OMe>OEt>OPr^i>OPh$. As in other series, steric similarity therefore appears to be an important requirement for activity. For these compounds, the *trans* substituted cyclopropane configuration is more effective than the *cis*. Activity is diminished in the corresponding thioether ($R^1=H$, $R^2=SMe$) and in a case when $R^1=Ph$, not H.

Insecticidal selectivity. Screening of recently developed compounds using *Chrysoperla carnea* larvae and *Myzus persicae* adults reported last year (*Rothamsted Report for 1984,* 103) has been extended and now includes susceptible (S) and resistant (R_2) strains of the latter. The natural ability of *C. carnea* to metabolize esters (especially *cis* forms) was not reflected in any significant differences between their susceptibility to ester and non-ester pyrethroids. Similarly, in spite of the established ability of R_2 *M. persicae* to attack certain esters, there was no general difference in resistance factor for the two groups. However, the accuracy of the bioassay is insufficient to detect minor differences.

Analysis of isomers by chiral HPLC. The separation of pyrethroid diastereomers by HPLC was readily achieved on a cyano-bonded column, eluted with 0·2–1·0% diethyl ether in hexane. For separation of enantiomers (particularly useful for the analysis of technical preparations and formulations, where assessment of biologically active isomers is crucial) a chiral column (Pirkle type 1-A ionic) based on *N*-(3,5-dinitro-benzoyl)-R-phenylglycine proved effective. Satisfactory resolution was obtained for compounds with a variety of acid and alcohol moieties, and containing one to four chiral centres, by eluting with 0·02–0·2% propan-2-ol in hexane.

Diastereomers were always more readily separated than enantiomers, and *cis* isomers, where assigned unambiguously, eluted before *trans*. Within enantiomeric pairs containing no α-cyano group, the (1R) isomer was always eluted before the (1S). For α-cyano esters, this was sometimes so (e.g. fenpropathrin) but sometimes not (e.g. WL 85871, a *cis* isomer pair of cypermethrin).

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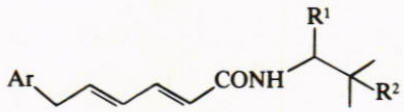

(III)

Ar=phenyl, 3,5-difluorophenyl, dibenzofuran-3-yl, 5- or 7-halonaphth-2-yl.
R^1,R^2=H or CH_3.

Insecticidal amides. Work based on the structure-activity relationships reported last year has led to active compounds, with the structures shown (III). Though less active than some recent insecticides such as the synthetic pyrethroids against both houseflies and mustard beetles, they are as effective as many other recognized insecticides in topical application tests. In addition, these compounds are consistently more effective against houseflies bred to contain *super-kdr* as the only resistance mechanism than against susceptible flies. *Super-kdr* flies, up to 400 times more resistant against pyrethroids and DDT, are up to four times more *susceptible* to *N*-alkylamides than the pyrethroid-susceptible strain. Such negative cross-resistance may prove to be a powerful weapon in countering *kdr* resistance, since it raises the possibility of selecting against *kdr*, once acceptable insecticides of this class have been developed.

Differential toxicity of *N*-alkylamides between S and *super-kdr* strains was maintained after the flies had been treated with synergist. Furthermore, flies with *super-kdr* were knocked down faster in spray tests by *N*-alkylamides than susceptible flies. Therefore the negative cross-resistance between pyrethroids and *N*-alkylamides operates throughout the course of the toxic response.

A similar comparison between the responses of susceptible and resistant aphids (*M. persicae*) was not possible because the *N*-alkylamides were insufficiently active against this species.

(Chemical work: Janes; Baydar, Cayley, Johnson, Khambay, Simpson. Biological work: Sawicki; Farnham, Stevenson; Morrison, Peel, Robertson, Smart)

Compounds influencing behaviour of invertebrates

The objective of this work remains the development of behaviour-controlling chemicals as an alternative to pest control agents active by toxic effects, in order to minimize environmental pollution and to provide materials useful against insecticide resistant pests. Development of the full potential of behaviour-controlling chemicals in agriculture depends not only on basic studies in identification and synthesis of active compounds, but also on directing such chemical studies to problems encountered during field trials.

Aphids. The activity of compound IVa, a 1,4-cycloaddition reaction product of the aphid alarm pheromone (*E*)-β-farnesene, in decreasing colonization of plants and virus disease transmission by aphids, already demonstrated in the laboratory (*Rothamsted Report for 1982*, Part 1, 126), now extends to the field. In autumn sown barley, compound IVa, applied electrostatically, reduced incidence of barley yellow dwarf virus and increased the yield from 6·47 to 7·28 t ha^{-1}. This is the first successful field use of a behaviour-controlling chemical against an aphid-borne virus disease. However, multiple treatments at high levels (1 kg ha^{-1}) were necessary, so more active and persistent compounds are being sought. (With Plumb, Plant Pathology)

Major modification to the homogeranyl structure (R^1 in IVa), originating from (*E*)-β-farnesene, gave compounds IV (R^2=undecanyl, R^1=undecanyl, nonanyl, 4-methylpent-3-enyl or phenyl) which were inactive in laboratory tests at the highest dose. Compounds

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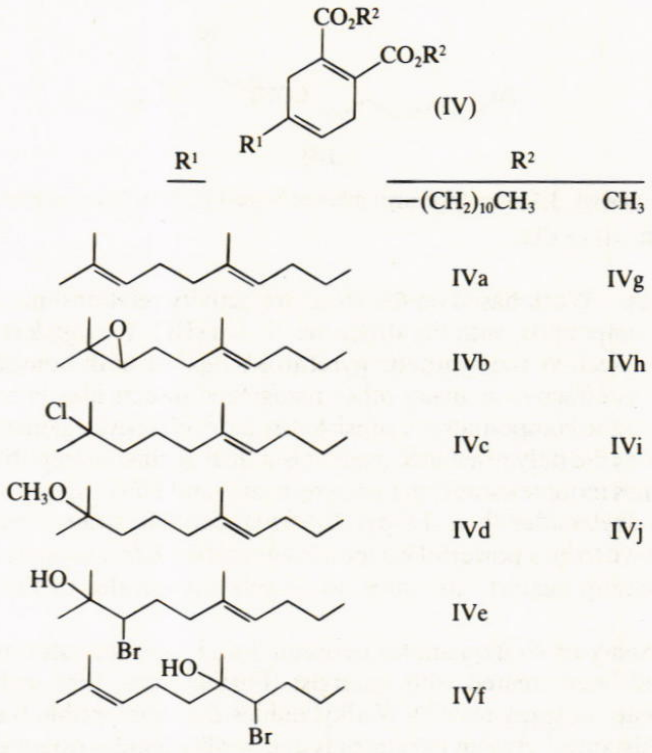

IVb–f with stereochemistry and charge distributions more similar to those of IVa, were also inactive. Replacement of undecanyl in IVa with methyl (IVg) removed activity, but surprisingly a similar modification to structures IVb–f, giving IVh–j, resulted in detectable activity for compounds IVh and IVi.

One of the most promising plant-derived antifeedants in laboratory tests against aphids is (−)-polygodial, so larger quantities are being accumulated for field trials. Undried water-pepper plants (*Polygonum hydropiper*) grown commercially on a large scale from seed obtained by glasshouse cultivation, were extracted directly with liquid CO_2 at 5°C to yield 0·02–0·04% of (−)-polygodial as a 20% extract (HPLC analysis on Spherisorb nitrile). Synthetic (−)- and (+)-isomers of polygodial can be resolved by HPLC with a chiral β-cyclodextrin bonded column. Extracts active against aphids have been obtained from the bugle, *Ajuga remota,* and related species. (With Dr A. Hassanali, International Centre for Insect Physiology and Ecology, Nairobi, and Dr L. E. Fellows, Royal Botanic Gardens).

Synthetic aphid alarm pheromone has been used in the field to improve contact action of insecticides (*Rothamsted Report for 1984,* 104). The synthetic pheromone, applied with an electrostatic sprayer in a glasshouse, has now been shown to improve the kill of aphids on *Chrysanthemum indicum* by *Verticillium lecanii* spores (Vertalec, Microbial Resources Ltd). Numbers of healthy aphids remaining on untreated, *V. lecanii* treated and *V. lecanii*+pheromone treated plots were in the ratio 9:3:1. (With Mrs S. H. Hockland, ADAS Reading). These results are particularly encouraging because the predominant aphid was *Aphis gossypii,* which does not respond well to the synthetic pheromone.

The turnip aphid *Lipaphis erysimi* can also give a poor alarm response with synthetic pheromone, (*E*)-β-farnesene. However, crushed aphids containing a similar amount of (*E*)-β-farnesene give a high response shown to be due to interaction with plant derived compounds in the aphid. As expected, volatiles isolated by vacuum distillation from the host

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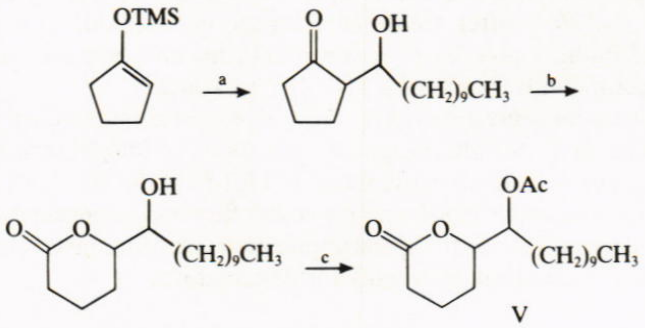
plant turnip (*Brassica rapa*), only slightly active alone, also gave a high response in the alarm bioassay when applied together with (*E*)-β-farnesene.

Total volatiles from aphid and from turnip, each analysed by capillary column gas chromatography (GC) coupled with electrophysiological recordings using a single cell preparation of the insect antenna, showed activity in several peaks. The compounds were identified by GC coupled mass spectrometry as a series of isothiocyanates, (RNCS; R=allyl, but-3-en-1-yl, pent-4-en-1-yl, 2-butyl). These compounds all substantially increased the response to (*E*)-β-farnesene. The most active compounds, as determined by dose response measurements were the allyl and pent-4-en-1-yl analogues. Although an aqueous solution of (*E*)-β-farnesene was inactive, addition of allyl isothiocyanate (the most readily available compound) gave an excellent response, and represents the first successful aqueous formulation of this pheromone.

Lepidoptera. Five photolabile propheromones were tested in the Punjab region of Pakistan for mating disruption against *Earias insulana* and *E. vitella* by spraying directly onto the cotton plants. Three gave lower trap catches than controls for up to ten days but the stabilized (black) microencapsulated pheromone was more effective. (With Macaulay, Entomology)



Synthesis of mosquito pheromone

a, $OCH(CH_2)_9CH_3$, $TiCl_4$; b, MCPBA, KF; c, Ac_2O, pyridine.

Mosquitoes. A new synthetic route to the mosquito oviposition pheromone (V), involving a Baeyer-Villiger reaction to form the lactone ring, gives a product containing all four isomers (i.e. racemates of *erythro* and *threo* isomers), but it is no less active than the natural isomer (−)-(5*R*, 6*S*)-6-acetoxy-5-hexadecanolide. A formulation of synthetic pheromone in effervescent tablets has been successfully employed in field tests in Sri Lanka. (With Dr B. R. Laurence and Mrs M. M. Pile, London School of Hygiene and Tropical Medicine)

(Pickett; Blight, Cayley, Dawson, Griffiths, Mudd, Smith, Wadhams, Woodcock, Zhang)

Resistance to insecticides

Work reported this year represents a systematic investigation of the biological properties of variants of *kdr*, the major mechanism of resistance to pyrethroids in the housefly, using a variety of techniques, because analogous mechanisms are known to operate in many other economically important instances of resistance. Similarly, resistance in aphids, caused by increased esterase production, is being studied both fundamentally, to establish the molecular basis of enzyme regulation, and in the field, to monitor the dynamics of this resistance.

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Relationship between levels of resistance and structure of pyrethroids in topical application assays. A study of the response of several strains of houseflies of different geographical origins with *kdr* or *super-kdr* has demonstrated that whereas *kdr* confers only slight to moderate resistance to the pyrethroids with no clear indication of relationship between the structure of the compounds and levels of resistance, the levels of resistance in strains with *super-kdr* can be correlated with the nature of the alcoholic moiety of the pyrethroid molecule. In *super-kdr* flies, resistance is strongest to compounds containing the α-cyano-3-phenoxybenzyl alcohol, such as deltamethrin, cypermethrin or fenvalerate, and weakest to compounds containing cyclopentenolones (S-bioallethrin and the pyrethrins).

Two variants of *super-kdr* have been identified on the basis of their cross-resistance characteristics to the pyrethroids.

Effect of *kdr* and *super-kdr* on the knockdown activity of the pyrethroids in spray tests. How *kdr* and *super-kdr* affect the knockdown activity of natural pyrethrins, bioresmethrin, permethrin and deltamethrin was investigated by spraying susceptible, *kdr* or *super-kdr* flies in a Kearns and March spray chamber, and establishing for each strain and compound the EC50 time curve for the first 30 min and LC50 after 24 h.

At the strongest concentration, the time-lag between treatment and 50% knockdown was shortest for natural pyrethrins, longest for permethrin. However, once knockdown had begun, deltamethrin knocked down fastest, and was the most toxic pyrethroid both during the first 30 min and 24 h after treatment. Piperonyl butoxide (pyrethroid/synergist, 1:8 w/w) delayed initial knockdown by the pyrethrins and bioresmethrin, but increased toxicity of both compounds 30 min and 24 h after treatment.

Although stronger concentrations of pyrethroids were needed to elicit knockdown of *kdr* and *super-kdr*, neither factor altered greatly the speed of knockdown by the pyrethroids tested, and only *super-kdr* significantly increased KT50 at the strongest concentration.

At kill end-point, resistance in *kdr* and *super-kdr* flies was strongest to deltamethrin, and weakest to natural pyrethrins, as in topical application tests, but the levels of resistance were less than by topical application, especially for deltamethrin.

Effect of *kdr* and *super-kdr* on the response of houseflies to residues of pyrethroids. Houseflies in population cages (*Rothamsted Report for 1984,* 106) show marked avoidance, greatest in susceptible flies, to high strength deposits of commercially formulated permethrin. Since this behavioural response is likely to influence the rate of selection of *kdr* and *super-kdr* by permethrin residues in the field, survival schedules of susceptible, *kdr* and *super-kdr* flies in population cages treated with permethrin residues are compared with kill in residue tests in which adults are confined in close proximity to permethrin residues. These tests also provide a comparison of the levels of resistance in *kdr* and *super-kdr* flies obtained by residue, spray, and topical application tests, data necessary for improved characterization of these important factors of resistance to pyrethroids.

Negative cross-resistance between the pyrethroids and insecticidal amides in flies with *kdr* and *super-kdr*. (See section on Insecticidal Amides, above).

Analyses of housefly sex-determining mechanisms. Collaborative work with Drs P. G. and M. Rubini, of the University of Pavia, Italy, has established that the unusual sex-determining mechanism prevailing in housefly populations near Rothamsted (*Rothamsted Report for 1981,* Part 1, 128) involves an interaction of a dominant female determinant (F), an X-linked male determinant (X^m) and a rarer male determinant on autosome 3 (MIII). Most flies of both sexes are morphologically XX and homozygous for X^m. Although widespread in south-east England, this mechanism is not typical of the country as a whole. Instead there is a

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gradual decrease in the frequency of F, X^m and MIII, and a concomitant increase in the typical male determining Y chromosome, on moving north, east and west of this region.

It appears that this radial cline in sex-determinants represents a transient polymorphism caused by a recent and continuing invasion by novel sex-determinants into populations originally having the XY mechanism. Whether such rapid evolutionary changes are related to the spread of insecticide resistance in houseflies, as some authors have suggested, is still unclear.

Biochemical studies of *kdr* in houseflies. Arrhenius plots of membrane-bound acetylcholinesterase suggested that phospholipids modulate its properties differently in susceptible and *kdr* housefly strains. This was supported by preliminary measurements of the fatty acid composition in the membrane fraction from fly-heads, which indicated a higher proportion of saturated fatty acids in *super-kdr* than susceptible flies (*Rothamsted Report for 1981,* Part 1, 130). However, more extensive analysis revealed no statistical differences between strains, and also demonstrated that large changes in the bulk composition of the membrane fatty acids arising from dietary changes had no effect on resistance. The phospholipid differences inferred from Arrhenius plots are therefore likely to be a consequence of changes in the lipid microenvironment of the membrane proteins.

Genetic basis of insecticide resistance in *Myzus persicae*. Insecticide resistance in *M. persicae* arises from increased levels of the mRNA coding for an insecticide-degrading carboxylesterase (E4), possibly due to gene amplification (*Rothamsted Report for 1984,* 107). To enable further studies of the molecular basis of this resistance mechanism, poly A^+ RNA was extracted from highly resistant aphids, size-fractionated and used to synthesize cDNA in the vector plasmid pUC8. The plasmids were then used to transform *E. coli* (JM83) and transformants selected by their resistance to ampicillin and their lack of catabolism of X-gal. A preliminary screen of the resulting cDNA library of 1500 clones, using ^{32}P-poly A^+ RNA from both resistant and susceptible aphids, identified a number of clones that hybridized more strongly to resistant mRNA than to susceptible, and are therefore candidates for isolating the E4 gene, via recombinant plasmid DNA.

Radiolabelled cDNA clones corresponding to the E4 message will be used to analyse for the presence of gene amplification associated with insecticide resistance. In preparation for this, high molecular weight DNA has been isolated from a number of aphid strains with varying degrees of resistance and E4 content. These DNA preparations are also being used in the construction of aphid genomic libraries using bacteriophage λ (EMBL4) DNA as a vector. (With B. G. Forde, Biochemistry)

Adaptation of a serological technique for the identification of resistant *Myzus persicae*, and its use in the field. An immuno-plate assay (*Rothamsted Report for 1984,* 106), which relies on the immunological trapping of E4, was modified for use as a field surveying technique. Resolution between the three most common variants in the UK, susceptible (S), slightly resistant (R_1) and very resistant (R_2), was improved and quantified. Further refinement has also enabled the differential detection of resistance levels above R_2 which are occasionally found in the field. The development of a multi-way homogenizer capable of simultaneously homogenizing 96 aphids within an immunoplate allows the analysis of well over 1000 aphids per day.

This technique was used in conjunction with field cage studies on the differential rates of selection for the different levels of resistance achieved by repeated spraying with the commercial formulations 'Aphox' (pirimicarb; carbamate), 'Metasystox' (demeton-S-methyl; organophosphate) and 'Decisquick' (a mixture of deltamethrin; pyrethroid and heptenophos; organophosphate). All insecticides, particularly 'Decisquick', selected

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rapidly for resistance, with R_2 frequencies after three sprays approaching 1·00. The initial starting frequency of R_2 (from 0·02 to 0·20) had little effect on the final outcome of selection although it altered its pattern of progress.

Local field surveys, using the immuno-plate assay, have been continued to attempt to further elucidate the factors governing the distribution of resistance in the field. (With H. D. Loxdale and C. P. Brookes, Entomology)

Insecticide resistance in *Phorodon humuli*. Field gathered damson-hop aphid (*P. humuli*) resists a wide range of insecticides, and as with *M. persicae* this is associated with increased esterase activity. To characterize further the biochemical basis of this association, the antiserum to E4 from *M. persicae* was tested for cross-reaction with homogenates of susceptible and resistant *P. humuli* (provided by Mr C. Furk, MAFF Harpenden Laboratory). Protein-staining of 'rockets' on Laurell immunoelectrophoresis gels was weak, but the esterases retained their activity after reaction with the antiserum (as previously found with *M. persicae*) so providing a more sensitive detection system and also establishing that the cross-reacting antigen(s) were esterases. The homology thus established in these enzymes between the two species showed clearly that the greater esterase activity in *P. humuli* also arises from increased esterase protein as opposed to a mutant enzyme form.

Insecticide resistance in *Aphis gossypii*. Some strains of the cotton aphid (*A. gossypii*) have become strongly resistant to pirimicarb on chrysanthemums in UK glasshouses, and some also show weak resistance to heptenophos and diazinon (C. Furk, personal communication). Four resistant strains were shown to have electrophoretically distinct, and more-active, esterases than the susceptible strain, but no causal relationship could be established from studies of insecticide binding or metabolism by these esterases. Furthermore, there was no cross-reaction with the antiserum to E4 from *M. persicae*. However, the acetylcholinesterase of pirimicarb-resistant strains was extremly insensitive to this insecticide (as previously reported by Silver (1984) Ph.D. thesis, Reading University), and in a strain additionally resistant to organophosphorus compounds, the enzyme was also less sensitive to inhibition by diazoxon and heptenophos.

(Sawicki; Dand, Denholm, Devonshire, Farnham, Moores, Perryman, Searle, Stribley, Venning, White, with ffrench-Constant, Entomology)

Systems for applying novel and conventional crop protection agents

Solutions for many pest problems depend on a combination of appropriate control agent and application technique. This is particularly true for novel agents (see earlier section on Compounds Influencing Behaviour of Invertebrates).

Performance of electrostatic spray systems. The performance of the prototype electrostatic equipment, descibed in earlier Reports, has been further evaluated. Collaborative work on insecticides (with Broom's Barn) and fungicides (with Plant Pathology and the Potato Marketing Board) are reported in other sections.

Early timing of electrostatic sprays may overcome difficulties in penetrating dense crop canopies with both herbicides and fungicides. The effects of early post-emergence sprays with chlorsulfuron plus metsulfuronmethyl ('Finesse') or late post-emergence sprays with 'Finesse' plus isoproturon (as 'Hytane') were investigated using electrostatic and hydraulic sprayers on two sites, one containing mainly grass weeds, the other containing grass and broad-leaved weeds. All treatments gave large increases in yield. The timing and choice of herbicide were more important than the type of sprayer. Although all applications of 'Finesse' gave almost complete control of broad-leaved weeds, except cleavers, early

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applications gave more effective control of grass weeds than late but addition of 'Hytane' to the late spray gave even better control.

The effects of varying the timing of different doses of electrostatically applied fungicides were studied in spring barley, sprayed with fenpropimorph against mildew at Zadoks growth stage 31, Zadoks growth stage 49, or both. In this trial, also, the main effects were caused by differences in regime rather than sprayer type. The Jumbo electrostatic sprayer and the standard hydraulic sprayer gave similar results at each spray time and dose; applications at the full recommended dose and one-third dose differed little; the late sprays gave the poorest, the early sprays intermediate and the early plus late sprays best control of barley mildew. Some saving of chemical was achieved with the early plus late sprays (at one-third recommended dose each time) which gave reliable control of mildew and yield increases of 26–33%; such divided treatments are easier to achieve with conveniently low volume electrostatic equipment.

(Cayley, Griffiths; Etheridge, Goodchild, Lewthwaite, Pye, Scott)

Pesticide distribution in plant and soil

A basic requirement in the understanding of pesticide action is knowledge of how efficiently the compound reaches the site at which it is effective, so studies on the relationship between distribution and chemical structure in two important systems, plants and soil, continue. This year, emphasis is on how the presence of ionizable groups affects such behaviour.

Translocation in plants. Measurements of the translocation in *Ricinus communis* of series of non-ionized and weakly acidic compounds have advanced our understanding of the mechanisms of phloem translocation. Non-ionized chemicals with a wide range of lipophilicity (log K_{ow} 0–3) enter the phloem sieve tubes freely by diffusion to give concentrations in phloem equal to those in the adjacent cells (K_{ow}=octanol water partition coefficient). Only chemicals that escape slowly from the phloem are retained sufficiently for long distance transport to occur, compounds that escape rapidly moving in the greater water flux in the xylem. These results support the intermediate permeability hypothesis of Tyree *et al.* (*Plant Physiology* (1979) **63,** 367–374) and show that only polar compounds with log K_{ow}<0 have the low permeability rates through membranes appropriate for translocation in phloem.

Weak acids with appropriate log K_{ow} also enter the phloem vessels, as the undissociated acid, and, because of the higher pH in phloem, dissociate there. Since the anions cross membranes very slowly, the acids can thus be accumulated in phloem by the ion trap effect. Concentrations of maleic hydrazide and some phenoxyacetic acids in leaf phloem were found to be up to ten times higher than those in the adjacent leaf tissue. These accumulations could be predicted reasonably well from the pH's of the relevant plant compartments, the pKa of the acid, the membrane charge and the ratio of the permeabilities of the membrane to the undissociated acid and its anion. This ratio was measured independently in barley roots and varied from $1{\cdot}8\times10^{2}$ for maleic hydrazide to 4×10^{5} for phenoxyacetic acids of intermediate lipophilicity.

Our results suggest that it is not necessary to invoke the existence of specific carriers for phloem loading of the compounds studied to explain their subsequent long distance transport on a similar physicochemical basis. Applying chemicals by injection into the petioles of *R. communis* allows translocation to be studied without the complication of cuticular penetration. To complement this work, the uptake and movement of 25 neutral and weakly acidic chemicals have been measured after application to the leaves of barley plants. The chemicals were applied in aqueous acetone containing 0·1% NP8 surfactant and parent compound was determined in the treated area, leaf above and below the treated area, other leaves and the roots. The results were consistent with those found in *R. communis*: neutral

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compounds were taken up rapidly and moved away from the treated areas to the upper leaf except for compounds with log K_{ow}>3 which were increasingly retained in the treated area; uptake of weak acids was fastest for those within the range log K_{ow} 0–3 and phloem movement showed a similar dependence on pKa and lipophilicity to that found in *R. communis*.

Pesticides in soil. Granular formulations of carbofuran and propesticides releasing carbofuran are being investigated at Broom's Barn as alternatives to aldicarb for the control of sugar beet pests. Release and transformation rates of several of these insecticides are being measured to provide data for modelling and to help relate properties of the chemicals to biological effectiveness.

The introduction of new weak acid herbicides has stimulated interest in their behaviour in soil. Adsorption and mobility on soil thin-layer plates was measured for six weak acids (pKa 3–3·8) with a range of lipophilicities (log K_{ow} 0·5–4·5) using soils from the Rothamsted and Woburn liming experiments. Adsorption decreased and mobility increased with increasing soil pH and with decreasing log K_{ow} of the acid. Good agreement was obtained between measured adsorption and that calculated from pKa, the adsorption coefficient of the acid and its anion and the pH, the soil surface behaving as though it is 2 pH units more acidic than the bulk solution. Behaviour of carboxylic acids and acidic amides was similar. Weak acids with log K_{ow} <3 will be very mobile in neutral soils under wet conditions.

Synthetic work. Much of the work of the group depends on the use of series of radiolabelled model compounds with appropriate pKa and partition coefficients. Labelled phenoxyacetic, phenylacetic and hydantoic acids, tetrazoles and oxazolidinediones have been synthesized using inexpensive labelled intermediates. A series of simple triazoles were made for work on volatile fungicides of potential value in control of take-all and for the measurement of partition coefficient and volatilization rates. The data can be used to calculate properties of fungicides with more complex structures.

(Briggs; Bromilow, Chamberlain, Evans, Nicholls, Rigitano, Williams)

Staff of the Department and the Chemical Liaison Unit

Sadly, Mavis Davies, P. Etheridge, R. E. Goodchild, A. R. Greenway, G. E. Gregory, P. J. Hulme, G. C. Scott, Clara Smith, J. H. H. Walters and M. R. Williams left, because their posts were declared redundant following cuts in funds available to the AFRC from Government. G. G. Briggs sought voluntary premature retirement. They have all made substantial contributions to the work and life of the Department and Unit and will be greatly missed. The Chemical Liaison Unit was disbanded and remaining staff were incorporated in the Insecticides and Fungicides Department. D. W. Hollomon, Jennifer A. Butters and Janice S. Clark were transferred to Long Ashton Research Station, and G. L. Bateman to the Plant Pathology Department to continue their work on plant disease control. D. A. Pulman resigned after 13 years and B. P. S. Khambay transferred from his temporary British Technology Group (BTG) post to replace him. A. D. Rice and H. S. Williams resigned and were not replaced.

M. Elliott completed 14 months as consultant to the British Technology Group and left to take up an appointment as visiting research scientist in the Pesticide Chemistry and Toxicology Laboratory, University of California at Berkeley. In other temporary posts financed by BTG, T. Javed replaced B. P. S. Khambay; A. Baydar, Caroline Beasant and Lorna A. Adams left, the last named being replaced by Louise M. Morrison, and N. D. P. Cosford worked for three months on chemical syntheses. B. M. Venning was appointed to work for three years with A. L. Devonshire on molecular biology of aphid resistance in an AFRC

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New Initiative post, and M. W. Rowland for two years with R. M. Sawicki on insecticide resistance in whitefly, financed by Ciba-Geigy.

R. Rigitano completed his research programme, was awarded his Ph.D. (London University) and returned to the Escola Superior de Agricultura de Lavras, Brazil. B. W. Simpson, Department of Primary Industries, Queensland, Australia, spent six months working with G. R. Cayley on separation of pyrethroid isomers. Among students working in the Department we were particularly pleased to welcome a voluntary worker from overseas—R. Hömke from West Germany.

J. A. Pickett made a number of broadcasts including several on British television when he discussed not only the work of the Department, but also more general aspects of crop protection policy. He gave a number of lectures and seminars including the Gooding Memorial Lecture to the Central Association of Bee Keepers and papers at meetings on animal behaviour at Oxford University, and on biologically active compounds of higher plants, organized by the Society of Chemical Industry. With colleagues he presented posters on plant-derived insect antifeedants at a Royal Society Conversazione, the Royal Show and a Society of Chemical Industry meeting on Biotechnology. With Margaret Blight he attended the Association of Applied Biologists meeting on the future of pheromones in applied entomology where both read papers. Pickett also organized a Society of Chemical Industry meeting on 'Crop Protection in Japan'. M. Elliott (chairman), N. F. Janes, R. M. Sawicki and A. L. Devonshire attended Neurotox '85. Devonshire also presented a paper at the Annual Meeting of the Entomological Society of America in Florida. J. H. Stevenson was appointed a Vice-President of the Royal Institution of Great Britain; he was chairman of the third symposium on 'Harmonisation of methods for testing hazard of pesticides to honeybees' (International Commission for Bee Botany) held at Rothamsted, attended the British Association meeting and, with Lesley E. Smart, presented a poster at the Association of Applied Biologists meeting on 'Field trials and data handling'. I. Denholm lectured at the Population Group Meeting in Manchester. G. G. Briggs (Session Organizer), R. H. Bromilow, G. R. Cayley, Avis A. Evans and P. H. Nicholls attended the British Crop Protection Conference—Weeds, the last two presenting a poster on movement of weak acids in soils. Staff also attended and contributed to a number of other meetings.

We take particular pleasure in reporting that the first award of the British Crop Protection Council's medal for outstanding services for British Crop Protection was given to our honorary scientist, C. Potter.

J. A. Pickett visited France (INRA) and Spain to discuss collaborative work on plant derived antifeedants and to present lectures at various institutes including the Ecole Polytechnique, Paris. The international recognition of the Department's work is also reflected in R. M. Sawicki's visits to France, Germany, Australia, Japan and the United States to discuss problems of insecticide resistance. M. Elliott (chairman), N. F. Janes, R. M. Sawicki and B. P. S. Khambay attended and the first three named gave papers (including one on behalf of J. A. Pickett) at the 190th American Chemical Society meeting in Chicago, some of which were repeated at a meeting on fluorine-containing pesticides at the Society of Chemical Industry in London. D. W. Hollomon visited Bayer in West Germany to discuss research programmes.

NEMATOLOGY DEPARTMENT

The work of the Department focuses on plant parasitic nematodes of greatest importance as agricultural pests in the UK and, with appropriate external funding, on some of those important in farming overseas. The Department's aims are to devise and develop various control methods including integrated control with nematicides, resistant cultivars, and more novel measures such as biological agents or interference with nematode behaviour in the field: these endeavours attempt to minimize pesticide application rates and identify

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approaches which will utilize agents with actions specific to the target organisms. Fortunately most major plant nematode pests are highly adapted parasites with specialized life cycles and host-parasite relationships, providing unique opportunities for novel methods of control, and current basic studies on nematode physiology and hatching mechanisms are laying the groundwork for control by interference with life cycles and normal behaviour. These studies are a main topic in this year's report. Some other aspects of the Department's work are also described more briefly but those on stem nematode, biological control, most aspects of host-parasite relations, taxonomy and pathotypes are omitted.

There has been a further loss of key scientific staff arising from cuts in government funding with consequent curtailment of the Department's programme. This has been offset by substantial commercial and MAFF funding for research on biological control agents, and ODA support for new work on biochemical characterization of root knot nematodes, a group of major importance in the tropics. A new SERC/CASE award with the Department of Pure and Applied Zoology, University of Leeds, provides for basic studies on the characterization of host specific cryptic nematode species, races and pathotypes, using biochemical and serological methods with *Ditylenchus* as a model. Nine of the 11 sections in a new edition of *Laboratory Methods for Work with Plant and Soil Nematodes* (ed. J. F. Southey, MAFF), which is the standard plant nematode laboratory manual worldwide, have been prepared or revised by members of the Department.

In various aspects of the work staff liaise closely with Plant Breeding Institute, Scottish Crops Research Institute, ADAS, Department of Agriculture and Fisheries for Scotland, Department of Agriculture for Northern Ireland, Commonwealth Agricultural Bureaux and a number of university departments; such collaboration is of increasing importance in maintaining the national effort in plant nematode research, to which the Department makes a major contribution.

Nematode hatching and physiology

Cyst nematodes characteristically reach a stasis point in life history when the juvenile, after development to the second stage, remains at low metabolic activity within the egg until hatch is triggered by specific stimuli.

Structure and function of egg membranes. The precursor to increased juvenile metabolism and subsequent activity leading to hatch of the potato cyst nematode species *Globodera rostochiensis* appears to be a change in eggshell permeability which is probably governed primarily by the inner lipoprotein membranes of the lipid layer. Following discovery of this layer in the eggshell of *G. rostochiensis* (Perry, Wharton and Clarke, *International Journal for Parasitology* (1982) **12,** 481–485), eggs of *Heterodera schachtii* (beet cyst nematode) and *H. glycines* (soya bean cyst nematode) have been examined using a recently developed cryofracture-fixation technique by which eggs, held between aluminium foil sheets and frozen in liquid nitrogen, are fractured and then thawed at 4°C in glutaraldehyde fixative before processing for transmission electron microscopy. The existence and ultrastructure of inner membranes which are usually closely apposed against the chitinous layer of eggshells of *H. schachtii* and *H. glycines* have been demonstrated. However, regional detachment was apparent, especially in older eggs of *H. schachtii.* In eggs from newly formed cysts of both species the membranes were predominantly tetra- and tri-laminate in structure but in eggs from older cysts of *H. schachtii* they were frequently single layered or absent. Mechanical abrasion by movement of unhatched *H. schachtii* juvenile and/or effects of products of the cyst microflora are possible explanations for this observation. The origin of the eggshell membranes remains uncertain. Examination of embryonating eggs of *H. schachtii* indicates that the membranes are autonomous and do not form a contiguous layer with the outer membrane of the juvenile. (Perry and Trett)

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The potential of the eggshell lipid layer as a novel target site for control agents can best be evaluated when the nature of the permeability change is understood. It is known that Ca^{2+} stabilizes biomembranes and the importance of Ca^{2+} and its probable structural role relating to eggshell permeability in *G. rostochiensis* has previously been reported (*Rothamsted Report for 1981,* Part 1, 161). Comparative studies have now been carried out on *H. schachtii*.X-ray microanalysis shows that eggshells of this species contain Ca^{2+} which is inaccessible to chelating agents and which is therefore thought to be located on the lipid layer. The eggshell Ca^{2+} content was significantly decreased when opened eggshells were treated with both organic and inorganic artificial hatching agents but was not diminished by treatment with hatching-inactive cations. These cyst nematode hatching agents probably induce structural change in the membrane and hence alter eggshell permeability by binding to, or replacing, membrane-bound cations.

Active root diffusate, which contains free Ca^{2+}, causes eggshells to bind additional Ca^{2+} indicating that new binding sites become available as a result of structural change in the membrane. One point of difference between *G. rostochiensis* and *H. schachtii* is that, whereas treatment of *G. rostochiensis* eggshells with decationized host root diffusates effected a decrease in Ca^{2+} content (Clarke and Perry, *International Journal for Parasitology* (1985) **15,** 511–516), a similar decrease was not detected when *H. schachtii* eggshells were treated with decationized beet root diffusate (DBRD). Perhaps under the conditions used, DBRD either displaced an insufficient proportion of Ca^{2+} to be detected or did not, in fact, displace Ca^{2+} from its binding site in the eggshell but bound to it to form a ternary complex which modified eggshell permeability. (Perry and Clarke)

Ascaris suum is a model system for investigating nematode hatching mechanisms (Clarke and Perry, *Parasitology* (1980) **80,** 447–456) and X-ray microanalysis has been used to examine the effects of various substances on the inorganic ion content of the eggshells. However, in contrast to the cyst nematodes studied so far, Na^+ rather than Ca^{2+} seems to play a major role in hatching. Eggshells isolated from untreated eggs of *A. suum* contained little Na^+ or Ca^{2+} but shells recovered from eggs hatched *in vitro* in hatching medium at 38°C contained substantial amounts of Na^+. Similarly, eggshells isolated from eggs treated with 0·1 M $NaHCO_3$ contained much Na^+. However, free shells treated with 0·1 M $NaHCO_3$ containing 0·2 M trehalose bound little Na^+. This is the concentration of trehalose found in the perivitelline fluid of *A. suum* eggs before hatch stimulation and it appears that the trehalose prevents the binding of Na^+ to the eggshell, suggesting that the Na^+ is not ionically bound by acidic groups. The $NaHCO_3$ may, therefore, be bound as an inclusion compound. There is evidence, in the form of blue–black iodine complexes, that the ascarosides of the eggshell lipid layer have distinctive binding properties comparable to those of cycxlodextrins. Tori formed by ascaroside headgroups in the presence of $NaHCO_3$ may provide channels for the escape of trehalose from within the egg prior to hatching. (Clarke and Perry)

A novel bioassay. The knowledge that a change in permeability of the lipid layer is one of the earliest events in the hatching sequence has enabled the development of a novel hatching bioassay technique using fluorescence microscopy. Preliminary experiments with *G. rostochiensis* have shown that when acridine orange was made up in solutions with various hatching activity (simultaneous hatching stimulation and stain exposure) marked differences were observed in the number of stained, unhatched juveniles. Over 85% of unhatched juveniles fluoresced after exposure to acridine orange in potato root diffusate (PRD), whereas only 35% were stained in acridine orange and distilled water; this relates closely to the percentage hatch obtained with cysts of the same batch in PRD and water respectively. Stain made up in zinc sulphate which has a lower hatching activity than PRD, gave 55% fluorescing juveniles. Acridine orange in PRD inactivated by heat treatment gave

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a similar percentage staining to that of distilled water treatment. Similar trends were seen using rhodamine although the percentages of stained, unhatched juveniles were consistently smaller. Routine hatching bioassays are time-consuming and require a large number of cysts. Their replacement by a test taking only hours to complete and using free eggs is desirable and this work indicates that this is feasible. (Perry, with Mr J. Feil, Queen Mary College, University of London)

Metabolic activity. It seems that PRD also stimulates juvenile activity directly (Clarke and Hennessy, *Nematologica* (1984) **30,** 206–212). Subsequent experiments in which hatching sequence was initiated then interrupted by changing the temperature (thus inducing juvenile quiescence) demonstrated that a cold period after initial PRD stimulation is unlikely to be followed by 'spontaneous' hatch on return to favourable conditions: additional stimulation is required. This reflects the importance of the metabolic component in the bimodal action of PRD and shows that, although brief exposures to diffusate will initiate the hatching sequence of *G. rostochiensis* (Perry and Beane, *Revue de Nématologie* (1982) **5,** 221–224), any interruption in the sequence which causes the juvenile to become dormant will necessitate further PRD stimulation before the hatching process can be completed. Such a process may protect the nematode from desynchronization of hatch with plant development when plant growth is checked by adverse conditions. (Perry and Beane)

Following a previous report of the effect of temperature on the physiology of potato cyst nematodes (*Rothamsted Report for 1975,* Part 1, 197) further work has established differences between *G. rostochiensis* and *G. pallida* that could be important in terms of competition between the two species. Studies with eight populations at six temperatures indicate that *G. pallida* seems better adapted to hatch at 10 and 15°C than *G. rostochiensis* and hatching of the former is more inhibited at 25°C. However, *G. pallida* had a much slower initial rate of hatch than *G. rostochiensis* at all the temperatures tested. Lipid utilization and mobility assessments indicated that persistence, as determined by a low rate of lipid utilization and a high percentage of active juveniles, was inversely proportional to temperature for both species between 10 and 25°C. *G. pallida* depleted its lipid reserves more slowly than *G. rostochiensis*; at 20°C *G. rostochiensis* had utilized 69% of its lipid reserves over 20 days whereas with *G. pallida* this figure was only 46%. It is considered that approximately 45% lipid depletion leads to a significant fall in infectivity.

The slower rate of hatch and greater persistence of *G. pallida* could result in this species securing the advantage of a growing root system leading to reduced intraspecific competition and greater fecundity of the females. (These characteristics may be partly responsible for the reduced efficacy of soil-applied nematicides in some circumstances.) However, the greater initial rate of hatch of *G. rostochiensis* may result in either a faster generation time or the exclusion of *G. pallida* from a stunted root system. It is likely that in mixed populations, *G. rostochiensis* is favoured by high densities and mild conditions and *G. pallida* is more likely to become predominant at non-damaging densities and lower soil temperatures. (Robinson and Perry with Dr H. J. Atkinson, University of Leeds)

Inheritance of hatching behaviour. Some potato cyst nematode eggs hatch spontaneously in the absence of potato root diffusate while others fail, in a given season, to hatch under that stimulus. These differences in behaviour may be determined genetically. To test this hypothesis newly produced cysts were placed in water and then several weeks later were transferred to potato root diffusate. Juveniles which hatched in the first two weeks in water or only after several weeks' exposure to root diffusate were kept separate and inoculated on to potato plants. The cysts produced were subject to the same procedure repeated twice at yearly intervals. Each year, the relative hatch of the two nematode lines in water and in potato root diffusate was compared. A relative increase in the numbers of juveniles hatching

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in water in the line selected for water hatch was observed in the second and third generations. By the third generation, in the line derived from nematodes hatching in water, significantly more individuals hatched in water than in root diffusate. These results suggest that hatching behaviour in relation to water and potato root diffusate is under relatively simple genetic control. In the field hatching response to potato root diffusate may thus be maintained by a simple selection effect. (Evans)

Hatching of *Heterodera cruciferae*. Some cyst nematodes lay a substantial portion of their eggs in an external gelatinous matrix (the egg 'sac') instead of retaining them within the body. The brassica cyst nematode, *H. cruciferae,* is an example and the internally and externally retained eggs may have different hatching behaviours; up to 37% of all eggs were found in the egg sacs attached to newly formed cysts. Egg sacs are not recovered when sampling field populations by standard methods. Some field cysts contained about 220 eggs so their egg sacs may have contained as many as 150 eggs. Studies of population dynamics and damage assessment will require a quantification of the contribution of eggs in egg sacs to overall population density, although it is possible that eggs in egg sacs hatch very rapidly or are not long lived.

Hatching in response to exposure to oilseed rape root diffusate was compared in egg sacs removed from newly formed cysts, field cysts (without egg sacs) and developing females from which the egg sacs had been removed. Juveniles emerged most readily from the egg sacs and very few emerged from the developing females. The optimum temperatures (of those tested) for hatching were 16°C for field cysts (31·4% hatch over eight weeks) and 20°C for egg sacs (52·2% hatch over eight weeks). The mean hatches at five temperatures over a period of eight weeks were 2·5, 40·1 and 21·6% for developing females, egg sacs and field cysts respectively. (Evans and Koshy)

General aspects

Control measures

Integrated control of potato cyst nematodes. Two new candidate oxime carbamate nematicides, provided by industry, were tested in field trials. In sandy loam soil at Woburn, heavily infested with *G. rostochiensis,* both compounds provided yield increases and nematode control similar to that with aldicarb, giving satisfactory results but in a peaty loam infested with *G. pallida* at Methwold Hythe, Norfolk, control (again similar to that with aldicarb) was less satisfactory. At Woburn aldicarb and two candidate organophosphate nematicides applied before a potato crop three years ago increased yields of susceptible potatoes significantly in 1985 but with a four-course rotation, nematicide application (aldicarb, oxamyl) was necessary before the second crop in order to maintain large yields. Although the soil was only lightly or moderately infested before the second potato crop was grown, aldicarb or oxamyl applied to the seedbed doubled tuber yields, compared with plots treated only before the first potato crop. This suggests either that light infestations are more damaging than previously thought or perhaps that many nematodes are able to migrate up from below the ridges in spring in untreated soil. New compounds providing substantially better control than the current major granular nematicides aldicarb and oxamyl have not been identified, nor do prospects for achieving good control by treating before alternate potato crops appear bright for longer rotations.

A modified vertical band technique for incorporting granular nematicides into soil was tested in silt loam with aldicarb at the commercially approved rate applied to the top 15 cm of soil in two passes of the vertical band applicator (*Rothamsted Report for 1982,* Part 1, 156) mounted on a Dutch harrow. This new vertical band technique, which does not involve subsequent lateral mixing of the granules with the soil (except that which occurs auto-

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matically in ridging up the soil during planting) allows fast, efficient and safe application of granular nematicide to the soil in spring. As no subsequent powered harrowing is needed, the technique is especially valuable for poorly structured soils but it is likely to be useful for most potato soils, except perhaps the very organic, in which more intimate mixing of the granules into the soil may be needed.

G. pallida is apparently increasing in importance in the UK. The predominant species in much of the ware land in Northern England, it is increasing in other areas as a result of widespread cultivation of Maris Piper potato, which is resistant to *G. rostochiensis* but susceptible to *G. pallida*. Four newer recommended cultivars, Cara, Kingston, Ukama and Pentland Javelin have the same resistance to *G. rostochiensis* and susceptibility to *G. pallida*. As yet there are no recommended, popular cultivars resistant to *G. pallida* or to both species of *Globodera*. Detailed studies of two fenland potato farms showed that *G. pallida* can replace *G. rostochiensis* as the dominant species in all parts of all fields under frequent cultivation of Maris Piper, despite the use of granular aldicarb or oxamyl. Indeed, field experiments have shown that in organic soils (at least) *G. pallida* increase on susceptible potatoes *cannot* be controlled by either of these nematicides at recommended doses. However, research aims to contain the threat, which *G. pallida* poses to quality potato production in irrigated soil, by combining the best new clones of potato resistant to *G. pallida* with nematicide treatments sufficient to prevent serious yield loss. Experiments on the control of *G. pallida* in Wilja potatoes at Woodwalton, Cambridgeshire and Methwold Hythe, Norfolk have shown that in irrigated peaty loam tuber yields are greater and nematode increase is reduced only by applying substantially more granular aldicarb. Optimizing the method of application had little effect. This suggests that either the extended period of hatching of *G. pallida* or the development of a second generation makes it more difficult to control than *G. rostochiensis* with non-persistent nematicides like aldicarb and oxamyl. Indeed, new cysts were observed on potato roots in October and tubers of susceptible cultivars dug at the end of October displayed new cysts full of eggs, indicating the presence of infective juveniles in the soil in mid-September. Whether these were survivors of the spring hatch or juveniles which hatched from the new cysts is not known.

All potato cultivars and candidate cultivars with resistance to *G. pallida* are only partially effective when compared with potatoes containing the major resistance gene to British populations of *G. rostochiensis* (see *Rothamsted Report for 1984,* 114–116). In collaboration with the Scottish Crop Research Institute (SCRI) and Plant Breeding Institute (PBI) advanced breeders' lines were again tested in field trials, together with cultivar Santé, of Dutch origin. Potatoes with partial resistance to *G. pallida* which yielded well were Santé, 'Heather' (11233ab22), 'Morag' (11305a2), 12290af20 and A27/23; 'Cromwell' (A27/20) and A25/11 were disappointing. Amongst susceptible cultivars, three new ones, Marfona, Diana and Kirsty yielded well in moderately infested soil treated with oxamyl. 'Heather' and 'Morag' appeared more tolerant of attack than other potatoes.

Control of beet cyst nematode. In the annual survey conducted by Brooms Barn, British Sugar and ADAS, the proportion of sampled fields in which beet cyst nematode (*Heterodera schachtii*) was detected has increased from 8·4% in 1977 to 33·5% in 1984. Because of this, and the recent relaxation in control of host cropping patterns on beet cyst nematode scheduled land, new emphasis is being placed on development of direct control measures. Aldicarb applied by a new vertical band reciprocating harrow technique to bands of soil 48·3 cm apart and 15 cm wide×15 cm deep, in which sugar beet was sown in spring, minimized damage to the crop by the beet cyst nematode in moderately and heavily infested soils, and greatly increased sugar yields. In moderately infested soil, as little as 1·7 kg aldicarb ha^{-1} applied in this way to the seedbed increased sugar yield by more than 20%. In heavily infested soil the same amount of nematicide increased sugar yields from 2 t ha^{-1} in untreated

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plots to 7·5 t ha^{-1}. Aldicarb at 9·9 kg ha^{-1} rotavated into the topsoil before sowing increased sugar yields to 10 t ha^{-1}. Control of the nematode was less when the reciprocating harrow was not used, granule incorporation being less uniform. (Whitehead, Tite, Penn, Fraser, Nichols and Westerdijk)

Nematicide trial with oilseed rape. A field, in which the oilseed rape cultivar Jet Neuf was grown in 1983/84, showed severe crop damage and had a final population density of 85 eggs g^{-1} soil of *H. cruciferae,* the brassica cyst nematode. This field was used to test the effects of autumn and spring applications of oxamyl, broadcast at 2·8 or 5·6 kg ha^{-1}, on nematode multiplication and yield of the oilseed rape cultivar Bienvenu. Even when oxamyl was applied at 5·6 kg ha^{-1} in both autumn and spring the increase in yield was small (3·29 compared to 2·92 t ha^{-1} in the untreated control). Much greater losses were found with cultivar Jet Neuf attacked by this nematode (*Rothamsted Report for 1983,* 115) and the good yield of untreated Bienvenu may be due to an ability to tolerate the nematode attack. No differences in nematode multiplication rate were found between treatments, the mean value being ×0·34. This may indicate that Bienvenu will sustain lower population densities of the nematode than Jet Neuf but the crop may have been harvested in the middle of a nematode reproductive cycle, so that most of the population was inside roots and the free cysts had low egg contents. (See also reports of factors limiting yield of oilseed rape in the Multidisciplinary Agronomy section of this Report). (Evans, Spaull, Russell and Harris)

Cyst nematode biology

Competition between potato cyst nematodes. *Globodera pallida* and *G. rostochiensis* can occur in mixed infestations and the effects of growing cultivars resistant to the latter are mentioned above. To investigate this and other cultivar effects in more detail ten microplots were established containing *G. rostochiensis* pathotype Ro1 and *G. pallida* pathotype Pa 2/3 (both from Woburn, Bedfordshire) in equal proportions. The same potato cultivar or clone was grown in each plot for seven consecutive years. The proportions of the two nematode species were then assessed by counting the numbers of new cysts formed on Maris Piper (resistant to *G. rostochiensis* Ro1) and Arran Banner (non-resistant), and by isoelectric focusing of nematode proteins (at DANI by Dr C. Fleming). The two methods of assessing species composition were in good agreement and showed that where Maris Piper was grown the population apparently became completely *G. pallida* (as expected); where early maturing cultivars were grown about two-thirds of the population was *G. rostochiensis*; where very late maturing clones were grown about four-fifths of the population was *G. pallida.* The preponderance of *G. rostochiensis* under early maturing cultivars was probably due to the faster hatching of this species, with the slower hatching *G. pallida* failing to complete a full generation before crop maturity. The preponderance of *G. pallida* where very late maturing clones were grown may have been due to slightly greater multiplication by *G. pallida* (without the penalty of an incomplete generation found on early maturing crops) or to reinvasion of roots by *G. rostochiensis* to begin a partial generation which was not completed. Persistent choice of early or late-maturing potato cultivars in a given area may therefore influence the preponderance of *G. pallida* or *G. rostochiensis* even when resistant cultivars have not been grown, but the amounts of either species introduced and the length of time over which introduction of one preceded the other are likely to have had even greater effects. (Evans)

Tolerance trials with potatoes and potato cyst nematodes. The problems of breeding potato cultivars with good resistance to *G. pallida,* and with ability to tolerate nematode attack,

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have been discussed previously (*Rothamsted Report for 1980,* Part 1, 155; *1984,* 116–118). To identify intolerant clones, breeders have to screen them in field experiments using small plots. A major constraint on trial design is the small number of tubers available from each clone in a large breeding programme: small plots consisting of few plants have to be used and relatively few clones can be tested in any one year. In order to maximize both the number of clones which can be tested and the amount of information on performance which can be gained from a small number of tubers, an unusual trial design from SCRI was tried. In this, plots consist of single plants (widely spaced to avoid competition from neighbours) with ten-fold replication, and are grown on a site heavily infested with potato cyst nematodes. Several similar trials were performed this year at other research centres, using clones supplied by SCRI. These included seven standard cultivars against which the performance of 19 breeders' clones were assessed. In a trial at Woburn, Bedfordshire, on a site which was infested with a range of population densities of *G. rostochiensis* Ro1, one clone (12243) was consistently identified as very tolerant, almost as tolerant as the best cultivar (Cara). This was confirmed at other centres, as was the identification of other tolerant clones. These results were achieved using only ten tubers of each clone and the approach has promise for assessing breeders' lines. Further collaborative trials are planned. (Evans, Russell, Harris)

***Interactions between potato cyst nematodes and* Verticillium dahliae.** The potato cultivar Pentland Javelin shows little evidence of attack by the wilt fungus *Verticillium dahliae* whether or not it is infected by potato cyst nematodes (*Rothamsted Report for 1982,* Part 1, 161). In comparison Maris Peer was little affected in the absence of potato cyst nematodes but showed the typical symptoms of wilting and unilateral chlorosis when nematodes were present; Maris Anchor had severe symptoms even in the absence of nematodes. Plants of these three cultivars were grown in culture on water agar and the roots inoculated at a range of distances from the tip with juveniles of *G. pallida,* to which none of the cultivars is resistant. Propagules of *V. dahliae* were added to some of the roots after 30 min and to others two, four and eight days later. Eight days after the introduction of *V. dahliae* root sections were taken for processing, sectioning and staining for light microscopy; other sections were taken remote from the point of inoculation and sub-cultured on water agar to assess the penetration of *V. dahliae* into vascular tissues. When *G. pallida* and *V. dahliae* were introduced together the amount of fungus penetrating the roots was greater than when *V. dahliae* was added alone; the distance from the root tip over which the fungus was able to penetrate the cortex to the stele was also increased. A delay between the addition of the nematode and the fungus resulted in less *V. dahliae* entering the stele of Maris Anchor or Maris Peer than when roots were inoculated with *V. dahliae* only.

Pentland Javelin roots were of relatively large diameter with up to 11 layers of thick-walled cortex cells surrounding a quickly-maturing xylem of large walled vessels; these present a considerable barrier to penetrating hyphae. Maris Peer has fewer layers of thinner walled cortex cells and its xylem vessels are of smaller diameter. Maris Anchor has few layers of cortex, especially near the root tip, and its slow maturing stele contains only four xylem poles compared with the five poles of the other two cultivars. All the cultivars produce lignitubers (lignin encapsulations of hyphal threads) in apposition to penetrating hyphae; by opening an invasion channel, nematodes enable hyphae to bypass this defence mechanism. Maris Anchor and Maris Peer each have a lignified hypodermis and both show a lignified hypersensitive response when invaded by the nematodes. This response spreads rapidly through the tissues and effectively seals off large areas of root from the exterior, so accounting for the resistance to the fungus induced by prior invasion of the nematode. Interactions between potato cultivars, potato cyst nematodes and *V. dahliae* are complex; in some circumstances the nematode facilitates the entry of the fungus but in others the nematode increases the plant's resistance to the fungus. (Storey, Evans)

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Potato cyst nematode host parasite relations. Fluorescence microscopy has been used to examine development and location of the hypersensitive response in susceptible and resistant potato cultivars invaded by potato cyst nematodes. Fluorescence at the point of entry of the nematode and along the track taken towards a feeding site was observed within 3 h of invasion. Fluorescence was a better indicator of the extent of the hypersensitive response than necrotic browning and was used to measure the distance nematodes travelled within the roots. Differences in measurements of fluorescence among the host-parasite combinations may be correlated with the degree of compatibility of the relationships or may be part of a more general wound response which varies among potato cultivars. Fluorescence was observed in cells surrounding the developing syncytium in incompatible cultivars. The extent of fluorescence which resulted from intracellular movement of the nematode was correlated with the distance travelled within the roots. Chemical and spectral analysis of the fluorescent tissues indicated that the fluorescence was due to the accumulation of phenylpropanoid compounds. (Robinson and Perry with Dr H. J. Atkinson, University of Leeds)

Development of* Heterodera cruciferae *on oilseed rape. Development of *H. cruciferae* juveniles occurred throughout the range 8–24°C on roots of oilseed rape, and proceeded faster the higher the temperature. The time taken to reach a series of developmental stages was noted for each temperature and assuming a basal development temperature of 5°C, the numbers of °C days above the basal temperature required to reach each stage were calculated. From invasion of roots to hatching of F_1 generation juveniles took an average of 680°C days, but only 210 were required for the first appearance of egg sacs on adult females. On this basis, two consecutive generations of *H. cruciferae* would be possible on autumn-sown oilseed rape in southern England (where 1100°C days above 5°C are available in the soil during the life of the crop compared to 850 in Scotland) but the second would only mature fully after harvest. In practice, however, overlapping generations probably occur due to flushes of hatching of juveniles (i) at sowing, (ii) when soils warm up after winter, and (iii) when the first generation completes its development. (Evans and Koshy)

***Effects of aldicarb on the ultrastructure of* Pratylenchus penetrans.** Following reports that exposure to low concentrations of oxime carbamate nematicides impaired orientation of plant-parasitic nematodes to known stimuli, but did not inhibit motility, the ultrastructure of the anterior sensilla of adult female *P. penetrans* was examined after exposure to aldicarb. Mature females were incubated in 1, 5 and 10 ppm aldicarb for 1, 12 and 24 h at 7°C and either processed directly for transmission electron microscopy or following 36 h incubation in artificial tapwater (ATW) (Greenaway, *Journal of Experimental Biology* (1970) **53,** 147–163). All treatments, except exposure to 1 ppm aldicarb for 1 h, induced increased densities of β-glycogen in the sarcoplasm of non-contractile portions of muscle cells. Abnormal 'vacuoles', probably representing leached, unsaturated lipid droplets, were present in the cytoplasm of several cells after exposure to 5 ppm aldicarb for 24 h and 10 ppm or 12 and 24 h. At these concentrations and exposure times, internal dendrite terminals within the amphidial sheath cell processes hypertrophied. These dendrites are thought to monitor sheath cell secretory activity. Two further effects observed in specimens treated with 10 ppm aldicarb for 24 h were the presence of large, electron-lucent granules in the amphidial sheath cell cytoplasm and denaturation of the sarcoplasm of the somatic muscle cells and anterior attachment processes of the stylet protractor muscles. With the exception of β-glycogen granule densities, none of these effects was reversed by 36 h incubation in ATW. It was concluded that disorientation effects probably relate to disruption of normal sheath cell secretory activity essential for stimulus transduction by the sensory dendritic processes. (Trett and Perry with Professor J. Green, Queen Mary College, University of London)

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Staff and visiting workers

D. H. Crump was awarded a Ph.D. and P. R. Burrows a B.Sc. Honours. A. J. Clarke retired after 30 years at Rothamsted, the last 17 in the Nematology Department working on chemistry of potato cyst nematode hatching factor and chemical aspects of hatching physiology; Susan B. Jepson left after completing a revision of root knot nematode taxonomy under contract to ODA. The posts of five staff were declared redundant. Of these, C. D. Green, Alison Spaull and Judy Hennessy have made long and major contributions to the scientific programme of the Department. All of the departed staff will be sorely missed. Four graduate and one ASO short term posts were obtained from new research contracts.

A workshop on Identification of Cyst Nematodes was held for ADAS and the Department hosted an AAB workshop on root knot nematodes. A. R. Stone visited centres in Portugal to advise on potato cyst nematodes and was a member of an EPPO Panel on the same species held in Paris; A. G. Whitehead visited INRA laboratories under the AFRC/INRA liaison scheme; K. Evans and B. R. Kerry were invited to the University of Kiel; B. R. Kerry attended an IOBC/WPRS meeting in Wageningen on integrated control of soil pests; A. R. Stone, B. R. Kerry, R. N. Perry and A. G. Whitehead were invited to the NATO Advanced Study Institute on Cyst Nematodes in Martina Franca, Italy.

Long term Visiting Workers in the Department included Dr P. K. Koshy (India: taxonomy and biology of cyst nematodes), Dr M. Trett (Queen Mary College: functional ultrastructure), Dr G. Storey (Luton College of Higher Education: potato cyst nematodes), Azucena Salazar (Spain: general nematology), D. Jovičić (Yugoslavia: general nematology) and C. E. Westerdijk (Netherlands: potato cyst nematodes); there were also several shorter term visitors from overseas.

PLANT PATHOLOGY DEPARTMENT

The aim of Plant Pathology Department's programme is to anticipate and respond to agricultural developments and to devise new methods of control based on a sound understanding of the systems involved. This report considers some aspects of work on 'new' crops such as sunflowers, 'new' diseases such as barley yellow mosaic virus transmitted by a root-infecting fungus, and black dot of potatoes, and new approaches to the control of 'old' disease problems such as potato virus Y and late blight. This illustrates the firm and innovative base of work in the Department which has helped to attract much outside funding, sadly at a time when permanent staff with much experience are being made redundant. In addition to the work reported here the Department contributes substantially to work on beans, wheat, barley, potatoes and oilseed rape that is reported under Multidisciplinary Agronomy.

Diseases of break crops

Oilseed rape

Early infection and control of light leaf spot. Despite earlier severe weather, on 31 January sporulating acervuli of *Pyrenopeziza brassicae* were found on 26% of plants of cv. Jet Neuf, 5% of cv. Darmor and 4% of cv. Bienvenu and 8%, 2% and 1% respectively of fully expanded leaves were infected. By 8 March dissection of tissues of Jet Neuf showed that sporulating acervuli were present in apical tissues deep within the crown of rosette-stage plants indicating that this infection was probably initiated before January. Subsequent sampling confirmed that young, unexpanded tissue of bracts, pedicels and buds on primordial branches in the axils of main stem leaves was infected long before symptoms were readily visible. Control of infection by prochloraz (as 'Sportak' at 1·25 litre ha^{-1}) was more effective when applied on 26 November than on 4 April and these sprays still had an effect in July.

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Growth regulators and disease. Shortening rape with growth regulators could increase infection, especially by splash dispersed pathogens, by changing canopy structure and crop microclimate and also by allowing greater use of nitrogen fertilizer. However, work with triapenthenol, a new triazole plant growth regulator, has shown that it has fungicidal activity against light leaf spot.

Triapenthenol sprayed on 17 April was compared with 'Terpal' (mepiquat chloride+2-chloroethylphosphonic acid), 'Terpal' plus a triazole fungicide (propiconazole as 'Tilt'), propiconazole alone and a non-triazole fungicide alone (prochloraz as 'Sportak') on cv. Jet Neuf. All treatments except 'Terpal' decreased light leaf spot. Untreated plots yielded 3·00 t ha^{-1}, after triapenthenol 3·28, after 'Terpal' 2·99, after 'Terpal'+propiconazole 3·49, after propiconazole 3·32, and after prochloraz 3·21 t ha^{-1} (SED=0·180). (Rawlinson; Church and Duckney)

Viruses of oilseed rape. In an experiment at Rothamsted, beet western yellows virus was detected by enzyme-linked immunosorbent assay (ELISA) or immunospecific electron microscopy (ISEM) in 21% of plants sampled in mid-April (before flowering) and in 34% sampled in mid-June (after flowering). Four times as much infection was detected in plots sown early (16 August 1984) as in plots sown late (6 September), and eight times as much in plots without insecticide as in plots treated in autumn with deltamethrin. A wide variation between replicates indicated a patchy distribution of infection. This, the effect of autumn-applied insecticide and the limited increase in infection during spring suggest autumn spread from scattered foci of infection established in early autumn. (Njuguna; Govier and Cockbain)

Sunflower

Diseases and yield. Eight replicates of six selected varieties, sown 10 April and harvested 30 September, tested the effectiveness of fungicides for control of *Botrytis cinerea*. Some early maturing varieties were particularly susceptible with more than 50% of the heads destroyed before harvest. Other varieties, however, had <5% of completely infected heads. Carbendazim+vinclozolin (as 'Bavistin FL' at 0·5 litre ha^{-1}+'Ronilan' at 0·75 litre ha^{-1} in 220 litre water) applied by hydraulic sprayer on 18 June, 8 August and 4 September failed to control *Botrytis* and had little effect on yield which ranged from 1·29 t ha^{-1}, from a *Botrytis* susceptible variety, to 4·80 t ha^{-1} from the best variety (SED±0·139). The APE 80 electrostatic rotary atomizer deposited more chemical than did the hydraulic sprayer on sunflower heads. One plot was infected with *Sclerotinia sclerotiorum* which destroyed the heads of affected plants. Forty-one varieties, many hybrid dwarf types, from France, Hungary, Rumania, Sweden, USA and USSR were grown in single plots of 3 m×4 rows with 50 cm row-spacing and a plant population of 80 000 ha^{-1}. Some varieties grew and yielded well (range 0·89 to 4·97 t ha^{-1}). Time taken to reach 50% emergence ranged from 23 to 46 days, mean height varied from 58 to 120 cm, anthesis took from 7 to 22 days, percentage heads infected with *Botrytis* prior to harvest ranged from 4 to 96%, and 17 yielded >3 t ha^{-1}. Yields of varieties sown later were generally less, ranging from 0·66 to 3·73 t ha^{-1}. (Rawlinson; Church and Duckney with Jones, Martin-Smith, Norrish and Turnell, Field Experiments, Cayley and Pye, Insecticides and Fungicides)

Diseases of grain legumes

Viruses of pea. White clover mosaic virus was detected at Rothamsted in July in combining peas showing a mild yellowish mottle. Incidence in one crop was about 7% but the mode of spread of the virus in pea is not known. In glasshouse tests two isolates were not transmitted by *Acyrthosiphon pisum* and *Sitona lineatus* allowed various acquisition and

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inoculation access times. The virus was not detected in grain lupins (*Lupinus albus*) grown alongside infected peas.

Lucerne vein yellowing virus, which is believed to be either an aberrant strain of bean leaf roll virus (BLRV) or a virus which depends on BLRV for transmission by aphids, was detected at Rothamsted in combining peas showing stunting and apical chlorosis. In glasshouse tests the virus was transmitted in a persistent manner by *Acyrthosiphon pisum* to lucerne seedlings which developed bright vein-yellowing symptoms and contained spherical virus particles about 30 nm in diameter (detectable by ISEM using an antiserum to BLRV). The virus was not transmitted by manual inoculation of phosphate buffer or phenol extracts.

Pea seed-borne mosaic virus, which is now regarded as established in the UK, was detected in 10% of seedlings grown from a sample of 'petit pois' pea seed sent in for disease diagnosis. In glasshouse tests, plants infected through the seed yielded 63% fewer pods and 84% less seed, and plants inoculated before flowering 8% fewer pods and 36% less seed, than uninfected plants. (Cockbain; Woods and S. E. L. Roberts)

Cereal diseases

Eyespot population studies. In autumn 1984 an experiment was begun on Meadow field, which had 97% MBC-sensitive and 85% wheat (W-) type isolates in the preceding wheat crop. Plots were left uninoculated or inoculated in autumn 1984 with either 95% MBC-sensitive+5% MBC-resistant W-type isolates; 5% MBC-sensitive+95% MBC-resistant W-types; 95% MBC-sensitive+5% MBC-resistant Rye (R-) types; 5% MBC-sensitive+95% MBC-resistant R-types. Fungicides applied in late autumn (GS 21) and spring (GS 31) were: carbendazim, prochloraz, carbendazim+prochloraz, compared with no treatment. In April, immediately after the second fungicide application, and in July, samples were assessed for eyespot and lesions characterized. Eyespot was well controlled by treatments containing prochloraz; carbendazim was partially effective and only in uninoculated plots. In April, isolate types were recovered from untreated and prochloraz-treated plots in approximately the proportions in which they were applied as inoculum or were present in the background population. In July in uninoculated, untreated plots the percentages of R-type (46%) and MBC-resistant types (26%) were greater than expected and MBC-resistant types had become predominant (59%) in untreated plots inoculated with the mainly sensitive R-type mixture. Carbendazim treatment increased the proportion of MBC-resistant types in all inoculum mixtures indicating rapid selection of, and subsequent disease development by, these types. By July the prochloraz treatment had decreased R-types by 37% compared with untreated plots, considerably less than for W-types (66% decrease). The frequency of R-types isolated from all untreated plots increased from 31% to 49% between April and July; MBC-resistance increased from 47% to 52%. No recovered isolates grew on agar containing prochloraz at 1 μg ml^{-1}. (Bateman; Fitt, Kilby, Creighton with Dr D. W. Hollomon, Long Ashton Research Station)

Ear diseases

Epicoccum *on ears of wheat*. Pinkish sporodochia, that superficially resembled those of *Fusarium* spp., were common on ears of mature wheat sampled on 12 August from the long-term, straw disposal experiment at Northfield (p. 172). However, no *Fusarium* spores could be found although spores typical of *Epicoccum purpurascens* were present. This fungus was also isolated from affected glumes plated on to malt agar. The spores of *E. purpurascens* are typically very dark but the mycelium in culture is often pigmented red and the pigment may diffuse into the substrate. On the specimens examined the underlying pigmentation was apparently evident because the sporodochia were immature and bore very few spores. It

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thus seems likely that the symptoms seen on the wheat ears can be attributed to *E. purpurascens*. (Jenkyn; Nabb with Christian, Soils and Plant Nutrition)

Take-all. The work this year has been concerned primarily with examining, evaluating and testing materials and methods reported to control the disease.

Fertilizers. Top dressings of different nitrogen fertilizers (200 kg N ha^{-1}) were applied to winter wheat crops at risk from take-all at Rothamsted. Rankings of the treatments for grain yield were different in 1984 and 1985 and there was no simple relationship between disease ratings and the yields obtained (range: 7·4–8·6 t ha^{-1}). Take-all ratings (max.=300) in July (Zadoks growth stage 69) were 220 (1984) and 233 (1985) with 'Nitro-Chalk' (N as ammonium nitrate) and 197 and 177 with ammonium sulphate. There were intermediate ratings for urea (219 and 221), ammonium chloride (211 and 181) and 'Nitro-Chalk' with potassium chloride (180, 1985 only). Any effects that chloride fertilizers may have had did not exceed those of ammonium sulphate, which confirmed previous observations in having least disease and relatively large, or best, yields. (R. J. Gutteridge; Bateman, Hornby)

'Baytan' seed treatment. Wheat grown in field plots from seed treated with 'Baytan' (25% triadimenol and 3% fuberidazole) at 2 g kg^{-1} had only slightly less take-all than untreated plants, irrespective of sowing date (10 September, 27 September, 15 October). Take-all ratings were greatest after early sowing (267 in untreated plots, and 253 with 'Baytan', in July). Premature ripening was significantly less in 'Baytan'-treated plots (29% whiteheads) than in untreated plots (43% whiteheads), but the grain yield difference of 0·54 t ha^{-1} was not significant. Last year (*Rothamsted Report for 1984,* 108) yield was increased significantly by 'Baytan'. 'Baytan' therefore has been beneficial where winter wheat was sown in early September and severe take-all developed. (Bateman; Hornby, R. J. Gutteridge)

Soil applied fungicides and biological control. The series of small plot experiments (*Rothamsted Report for 1984,* 128) continued on the same sites as last year, but with additional putative biocontrols (*Pseudomonas fluorescens,* strains 13–79 and 2–79 from the USA) and different methods of application (in-furrow and on seed for bacteria, drench and incorporation for nuarimol). Four strains of bacteria, two fungicides, four methods of application and two dates of application have now been tested at both Rothamsted and Woburn during three consecutive years. The bacterial treatments have had no consistent effects on take-all, yield or soil infectivity. The fungicide nuarimol has decreased take-all, more so when incorporated than when applied as a drench only, and it has been more effective at Rothamsted than at Woburn. The concentrations used, however, are uneconomic and sometimes phytotoxic. (Hornby; Bateman, Henden, R. J. Gutteridge, Payne, Statistics and Dr R. Campbell, University of Bristol)

Barley yellow dwarf virus (BYDV)

Autumn 1985. Infectivity was measured at nine of the same sites as in 1984. Although 1985 data are still incomplete, for all sites where a comparison is possible, the Infectivity Index is larger or much larger than in 1984 and the Infectivity Index for Rothamsted was 90 compared with a threshold value, above which treatment is advisable, of 50. Thus, it appears that crops sown in September should have been sprayed but spraying crops sown later would have been of doubtful benefit. (Plumb; Lennon, R. A. Gutteridge and Campbell)

Barley yellow mosaic virus (BaYMV)

Susceptibility of cultivars. Because resistant cultivars are likely to be the most practicable control strategy for the disease, one of the main aims is to develop screening tests and to

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explain the basis of resistance in some current cultivars. The virus has been routinely transmitted by mechanical inoculation, often using an artist's airbrush, and by using zoospores of the fungal vector (*Polymyxa graminis*), produced from sand cultures maintained in the glasshouse. The ranking of cultivar susceptibility was similar after the two inoculation procedures and generally reflected field experience. For routine screening, mechanical inoculation is more convenient and symptoms appear more quickly.

The susceptibility of several cultivars to the vector was tested using an automated system to flood the sand cultures with dilute nutrient solution for two periods of six hours each day and measuring zoospore production from the roots. There were no consistent differences between cultivars and it seems, therefore, that the resistance mechanism operates against the virus rather than the vector. Spring barley cultivars supported the growth of the viruliferous vector and some showed symptoms although, in the field, symptoms do not appear on spring-sown crops.

Effects of temperature. In the field, BaYMV symptoms usually appear in late winter and fade as the plants grow rapidly in late spring. It was therefore expected that the optimum temperatures for the growth of the vector and/or the development of virus symptoms would be fairly low. Experiments in a series of Wisconsin tanks, gave eight different root temperatures from 5 to 26°C. Using a viruliferous isolate of *P. graminis*, growth (as measured by zoospore production from the roots) was rather poor at all temperatures but there were large effects on virus symptoms, the optimum being 23°C, at which temperature symptoms began to appear within 18 days of inoculation. In a separate experiment, using larger pots, massive zoospore production occurred at temperatures between 10 and 20°C with production peaking and falling away more rapidly at 17 and 20°C than at 11 or 14°C. Few zoospores were produced at temperatures below 10 or above 20°C. Experiments using mechanical inoculation of several cultivars showed that symptoms were produced more rapidly at 23 than at 17 or 11°C. These unexpected results show that more information is needed to explain the pattern of symptom development in the field. (Adams; Swaby)

Purification. Published methods for the purification of BaYMV were modified by adding 0·1 M EDTA and 2·5% Triton X100 to the extraction buffer and by using a 30% CsCl solution for the final separation spin. This allowed consistent purification of two isolates of BaYMV. Antisera were prepared and used to study isolates of the virus from sites in England by ELISA and ISEM. All isolates so far examined are serologically identical to each other and to the BaYMV-M strain from Germany. (Jones; Adams and Swaby)

Potato diseases

Stem canker (*Rhizoctonia solani*). In 1985, seed tubers of three early cultivars (Arran Comet, AC; Estima, ES; Wilja, WI) and three maincrop cultivars (Désirée, DE; Maris Piper, MP; Pentland Squire, PS) were inoculated, when planted on 19 April, with cultures of *Rhizoctonia solani* grown on horticultural vermiculite (140 ml m^{-1} row). Small seed (50 g) was planted at 30 cm and large seed (130 g) at 51 cm, spacings that gave similar stem populations and tuber yields in uninfected crops at harvest.

Eleven weeks after planting, more shoots were pruned off from seed tubers of AC (38%) than WI (21%) or ES (14%), and stem canker (disease rating percentage) was more severe on WI (59) than AC (52) or ES (46), but was not affected by seed tuber size. The disease decreased tuber yields of AC and WI by 40%, and more from small (50%) than large seed (30%). Yields of ES were decreased by 23% (large seed) and 19% (small seed). Five weeks later, yield losses were larger from WI (30%) and AC (15%) than ES (8%) and with all cultivars were greater from large than small seed.

With maincrop cultivars, shoot pruning and stem canker were more prevalent on PS

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(32%, 54% respectively) than DE (28%, 42%) or MP (20%, 44%). Eleven weeks after planting yields were decreased most on MP (44%) and PS (37%) and especially from small seed. Yields of DE were decreased by 33% from small and large seed. By harvest in October, the disease had decreased yields most in crops from small seed (PS small 31%, large 18%; DE small 14%, large 4%) but with MP both small and large seed gave yields 14% less than uninfected. These yield results reflected decreases in fresh weight of foliage caused by the disease and, whereas numbers of aerial stems on infected AC, ES, DE and MP were increased, stem numbers of WI and PS were decreased, indicating that these cultivars were less able than others to compensate for shoot damage caused by the disease.

In another experiment, sprouted and non-sprouted seed tubers of cv. Désirée were inoculated with *R. solani* at 140 or 700 ml m^{-1} row. With both rates of inoculum, shoot pruning was more common with sprouted than non-sprouted seed (low 16 and 1%; high 21 and 5%) and with low inoculum stem canker was also more severe with sprouted (27%) than non-sprouted seed (21%). With high inoculum stems from non-sprouted seed were affected more than sprouted (47%, 40%). Inoculum at the high rate delayed shoot emergence and decreased fresh weight of foliage and tubers. However, inoculating non-sprouted seed at the low rate increased tuber yields by 13% (9 July), 6% (1 August) and 5% (5 September) and plant height and fresh weight of foliage were similarly increased. (Hide; Read, Sandison, Hall)

Black dot (*Colletotrichum coccodes*). In 1985, an experiment was planted at Rothamsted with seed tubers affected with black dot, or seed tubers without the disease uninoculated or inoculated with cultures of *C. coccodes* applied to the soil before covering. Half the plots were irrigated, so that soil moisture tensions were maintained wetter than −15 cm Hg (−20 kPa), from shoot emergence to the end of July. Some tubers had black dot at the end of July and disease incidence and severity increased gradually during August and more rapidly during September and October. Seed tuber infection increased disease during August but later gave only as much as disease-free seed, whereas soil-applied inoculum increased it on all dates sampled. Irrigation slightly decreased the disease up to mid-August but thereafter increased it and by late October disease severity (46%) was greater than from non-irrigated plots (37%). Black dot increased on tubers stored for nine weeks, and more on tubers held at 15 than 5°C whereas incidence did not change on tubers washed before storage which removed much superficial inoculum. (Read; Hide)

Late blight (*Phytophthora infestans*). In 1984 and 1985, plants in guard rows between plots (cv. King Edward) were inoculated with *P. infestans* at the end of July, and plots were sprayed with 'Fubol' 58WP (in 1984) or SL 291A (Ciba Geigy), a formulation containing metalaxyl (in 1985), using a hydraulic sprayer (200 litre ha^{-1}) or electrostatic sprayers (<10 litre ha^{-1}), either two days after inoculating ('early') or three weeks later ('late'). In 1984, similar fungicide deposits were achieved with the hydraulic and vertically mounted electrostatic sprayers and more was found on the upper (67–89 $\mu g\ g^{-1}$ fresh wt), than middle (42–45 $\mu g\ g^{-1}$) or lower leaves (15–23 $\mu g\ g^{-1}$). Compared with other sprayers, the Jumbo electrostatic sprayer deposited five times as much fungicide on upper leaves but only half as much on lower leaves.

Blight did not develop in 1984, but in 1985 the disease developed rapidly and on plots sprayed late almost 70% of the foliage was destroyed by 27 August. On this date, less than 1% of the foliage was affected on plots sprayed early and there were no differences in the amount of disease on plots treated using the different sprayers. This suggests that electrostatically charged rotary atomizers may give as good control of blight as hydraulic sprayers and have the advantage of using much less water. (Hide; Cayley and Lewthwaite, Chemical Liaison Unit and Pye, Insecticides and Fungicides)

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Improving the health of home-grown seed potatoes. Three treatment regimes, Present (P), Improved (I) and Maximum (M), were compared for controlling pathogens affecting seed potato production in a ware area. Before sprouting, foundation stock (1984) and super-elite (1985) seed tubers of cvs King Edward and Maris Piper were sprayed with the fungicides tolclofos methyl and imazalil at 0·24 kg and 0·01 kg t^{-1} respectively, using charged (M) or uncharged rotary (I) atomizers, or were left untreated (P). The charged rotary atomizer deposited 81% of the applied fungicide on the seed tubers compared with only 50% with the uncharged sprayer. Aphid control was by phorate (1·7 kg ha^{-1}) incorporated into the soil and by foliar sprays of pirimicarb (0·14 kg ha^{-1}). However, additional control of aphid-borne viruses was given to I and M regimes by roguing diseased plants and by hydraulic sprays of a mixture of mineral oil (71 ha^{-1}) plus cypermethrin (40 g ha^{-1}) applied either twice (I) or fortnightly throughout the growing season (M).

In 1984 and 1985, M plots yielded least, 10–25% less than P plots. Apparently the fungicide on seed tubers slowed emergence and the mineral oil sprays damaged the foliage. However, the yield of seed-sized tubers was little affected. August compared with September haulm destruction reduced yield by 30–40%, again affecting mainly the yield of ware-sized tubers.

Fungicide seed treatments greatly decreased infection of stem bases by *Polyscytalum* and *Rhizoctonia* in July. At harvest, silver scurf, black scurf and *Polyscytalum* were also decreased, and silver scurf and *Polyscytalum* were much less common on tubers lifted early than late.

Seed produced in 1984 was planted as a ware crop in 1985. Seed of both King Edward and Maris Piper from the P regime gave yields 5% less than AA Scottish which gave yields similar to those given by I and M seed. As well as more fungal pathogens, P seed of King Edward had 9% of tubers carrying PVY compared with 3% in I and 1% in M, and P seed of Maris Piper had 7% PVY and 2·5% potato leafroll virus compared with 2% PVY and 0·6% leafroll in I and 1% PVY and 0·6% leafroll in M seed. Early haulm destruction, which halved the incidence of PVY had no effect on the yield of ware. (Gibson, Hide; Read with Cayley, Chemical Liaison Unit and Harrington, Entomology)

Potato virus diseases at Rothamsted. When counts were made in early July, plots planted with Désirée, Maris Piper and Pentland Crown seed grown at Rothamsted in 1984 were free from virus infection. However, similar plots of King Edward had 16% PVY which must have spread during 1984 from the 1% tuber-borne infection in the seed crop even though the seed crop was intensively rogued and only limited spread was detected during 1984.

Aphids (*Myzus persicae*) were scarce in 1985 and only very limited spread of PVY was detected even in a King Edward crop close to a source of infection. (Govier)

Viruses and virus diseases

Beet cryptic virus (BCV). BCV double stranded RNA (dsRNA) extracted from a semi-purified preparation was inserted into plasmid pUC9 and cloned in *E. coli*. Two clones were identified, each being specific for one of the three dsRNA bands into which the preparation separated when electrophoresed in polyacrylamide gels. The clones were used as probes to detect infected plants. Some plants contained dsRNA that hybridized to both probes, others reacted to either probe but not both, suggesting that BCV is a mixture of more than one virus. (White; with Antoniw, Biochemistry and Dr J. Bol and Dr H. J. M. Linthorst, Biochemistry Department, University of Leiden)

Pathogenesis-related (PR) proteins

Distribution of tobacco mosaic virus (TMV) and PR proteins in and around local lesions. Local lesions on *Nicotiana tabacum* cv. Xanthi-nc plants inoculated with TMV

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were cut out, at intervals after inoculation, using a 14 mm diameter cork borer. Each leaf disc was further sectioned into concentric rings and a central disc using a series of cork borers of 10, 8 and 5 mm diameter. Each part was then extracted and analysed by ELISA for TMV and b_1 PR protein. The greatest concentration of TMV was in the centre of the lesion and decreased rapidly with distance from the centre. In contrast the b_1 protein first appeared in the centre of the lesion but from five days post inoculation the largest amounts were found in the 5–8 mm ring just outside the area with the highest concentration of TMV. This distribution is to be expected if PR protein is involved in, or closely associated with, restricting the spread of the virus. (White; with Antoniw, Biochemistry)

Effect of salicylic acid treatment on TMV and PR protein accumulation in local lesions. Lesions produced in Xanthi-nc leaves sprayed ten days before TMV inoculation with 3 mM salicylic acid were half the size of those in untreated leaves. Discs (14 mm diameter) centred on these lesions contained only 25% of the TMV present in discs centred on lesions of untreated leaves when analysed by ELISA. Unsprayed uninoculated leaves contained very little b_1 PR protein but this increased over 200-fold in salicylic acid sprayed leaves. Discs centred on TMV-induced lesions in salicylic acid sprayed leaves showed a 400-fold increase in b_1 over untreated controls, but TMV-induced lesions in unsprayed leaves contained even more. Thus the amount of b_1 protein induced is not simply an additive combination of salicylic acid treatment and virus local lesion formation but may be dependent upon the circumference of the ring of cells producing PR protein. (White; with Antoniw, Biochemistry)

Diseases of tropical crops. Diseases of uncertain or unknown aetiology form the majority of samples received from overseas for diagnosis. Melon rugose mosaic virus (MRMV) (*Rothamsted Report for 1984,* 132) continues to spread in PDR Yemen but work there has established that while the disease is severe in crops sown July–December, those sown in February–March are rarely infected. An immunodiffusion test using serum prepared at Rothamsted is being used to help with the identification of MRMV in PDR Yemen. (Jones)

Aerobiology

Rainfall intensity and duration and dispersal of *Rhynchosporium secalis* conidia. Simulated rain with an intensity of 12 mm h^{-1} or 6·5 mm h^{-1} was allowed to fall for 30 min on to horizontal, detached barley leaves infected by *Rhynchosporium secalis*. Most of the resultant spore carrying droplets were 400–800 μm in diameter but most of the spores were carried in droplets 800–1200 μm in diameter at 6·5 mm h^{-1} and 1600–2000 μm in diameter at 12 mm h^{-1}. Spore-carrying droplets were collected up to one metre from the source but less than 10% travelled more than 50 cm and spore deposition gradients were steep with half-distances of 5–8 cm.

During June and July 1985, spore-carrying droplets collected in a crop of winter barley (cv. Maris Otter) were mostly less than 400 μm diameter. Conidia were collected only when rainfall intensity was greater than 0·2 mm h^{-1} and the number collected increased with increasing rainfall intensity. (Fitt; Creighton with M. E. Lacey and McCartney, Physiology and Environmental Physics)

Airborne microorganisms associated with domestic waste disposal. In air samples at 16 domestic refuse transfer stations and landfill sites most microorganisms were found where waste was pulverized or moved in bulk by grab-crane, conveyor or bulldozer. Where conveyors loaded refuse into river barges, up to $5{\cdot}4\times10^5$ bacteria growing at 37°C and $3{\cdot}3\times10^6$ fungi growing at 25°C were found per cubic metre of air while transfer of refuse from storage bunkers to incinerators yielded $1{\cdot}5\times10^6$ bacteria (37°C) and $9{\cdot}2\times10^6$ fungi (25°C)

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m^{-3}. By contrast, numbers in a typical household environment were $2{\cdot}0\times10^3$ bacteria (37°) and $6{\cdot}3\times10^2$ fungi (25°) m^{-3}.

At landfill sites, concentrations of airborne microorganisms were greatest in the cabs of bulldozers spreading and burying refuse. At one site, concentrations were decreased to less than 1% of earlier measurements by the introduction of a containerized barge system and by air-conditioning the cabs. Seasonal variations occurred at all sites with peak concentrations of airborne microorganisms from August to October. The most common fungi isolated at 25°C were *Penicillium* spp., constituting 78% of the total, and at 37°C *Aspergillus* spp., with *A. fumigatus* forming 67% of the total isolates. (Lacey; Crook and Higgins)

Biodeterioration

The microflora of stored rape seed. Rape seed stored in Dewar flasks heated less than hay similarly stored. Spontaneous heating occurred only above $0{\cdot}98a_w$ (17·0% water content) with highest temperatures (15°C above ambient or 32·5°C after 20 days and 23°C above ambient or 47°C after 75 days) occurring in seed stored close to $1{\cdot}0a_w$ (20% water). *Aspergillus restrictus* predominated in seed stored with 0·77 to $0{\cdot}97a_w$ (7·8–14% water) together with some *A. glaucus* group at the higher a_w. These species were also found in the uppermost layers of seed bulks, initially at $0{\cdot}98a_w$ or above (>17% water) but *Penicillium* spp. predominated at greater depths until after 43 days storage. With $1{\cdot}0a_w$ (20% water or more), when *Penicillium* spp. were replaced by *Aspergillus terreus* and *Absidia corymbifera* there was heating to 23°C above ambient, when replaced by *Aspergillus versicolor*, *Scopulariopsis brevicaulis* and yeasts there was heating to 13°C above ambient and when *S. brevicaulis* and yeasts alone predominated there was still less heating. *Penicillium* species isolated included *P. aurantiogriseum (P. cyclopium)*, *P. brevicompactum*, *P. chrysogenum*, *P. citrinum*, *P. expansum*, *P. hirsutum (P. hordei)*, *P. implicatum* and *P. viridicatum*. (Lacey; Nabb)

Selective isolation of *Micropolyspora faeni*. *Micropolyspora faeni*, a thermophilic actinomycete from mouldy hay, is the chief source of farmer's lung antigen in Britain. Numerical taxonomic studies of isolates of *M. faeni* and related actinomycetes identified the ability to utilize hippurate as a nutritional character that could be used in media selective for this species. The hippurate medium of Gordon and Noram (*Journal of General Microbiology* (1968) **50,** 223–233) was modified by the addition of 20 g NaCl, 24 g agar, 5 ml 0·2% phenol red and 0·1 g cycloheximide (actidione) l^{-1} medium. With this medium, the numbers of *M. faeni* isolates from seven mouldy hay samples using an Andersen sampler/wind tunnel method was increased on average by more than ten times over isolation on tryptone soya agar (TSA). Hippurate agar plates were incubated for 9–12 days at 50°C, in polyethylene bags to prevent drying, to allow *M. faeni* colonies to develop sufficiently for counting and to grow through the thin film of bacteria that sometimes developed. Consistent counts of *M. faeni* on hippurate agar dilution plates were obtained if the plates were first covered with sterile 9 cm diameter, 0·22 or 0·45 μm pore size membrane filters. Inoculum was spread on the filters which were removed aseptically after about five days incubation. Plates were then incubated for seven more days before counting. (Lacey; Rose)

Staff and visiting workers

D. R. Henden retired after 40 years association with Rothamsted. Regrettably the posts occupied by D. H. Lapwood and J. M. Carpenter were made redundant; they contributed much to the Department's work during their service of 29 and 18 years respectively and will be missed. Pamela Evans retired early and R. H. Turner, Janette Isger and L. Beeken resigned. Mary White, Sally Higgins, Judith Wilson and I. Barker were appointed; G. L. Bateman transferred from Insecticides and Fungicides Department.

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Maryse Chabrol was awarded the degree of Docteur-Ingenieur and Janet Sandison H.N.C. in applied biology. Deirdre Rose and R. Parsonage worked in the Department as CASE award students. In addition 13 visiting workers and students, Tseng Shi-mai (People's Republic of China), Adelaide Fernandes (Madeira), Sylvie Nicoud, D. de Filippo (France), P. Nagy (Hungary), J. Njuguna (Kenya), K. Smidt (The Netherlands), C. Campbell, Katharine Duckney, Aurora Garcia, K. Grossett, Helen Gunn (AFRC Bursar) and Karen Kilby contributed to the work of the Department. Numerous staff played an active part as organizers, invited speakers and participants in many meetings at home and abroad. A. J. Cockbain and R. D. Woods each visited Nanjing University (People's Republic of China) in furtherance of links between our two Institutes on virus diseases. J. Lacey and J. F. Jenkyn visited The Bose Institute, Calcutta as part of a joint programme on aerobiology, supported by The British Council. The British Council also asked R. T. Plumb to visit Institutes in Maharastra, Andra Pradesh, West Bengal and New Delhi. R. T. Plumb also visited Sweden and J. Lacey Sweden, Norway and Finland and was an invited speaker at a Symposium on Mycotoxins and Phycotoxins in Pretoria, Republic of South Africa. C. J. Rawlinson and D. Hornby visited France with funds provided to support collaboration between AFRC and INRA. R. W. Gibson visited USA at the request of ICI and P. Jones was co-organizer of a session on virus identification in Rabat, Morocco, supported by the Overseas Development Administration. D. Hornby and J. Lacey continued as Chairmen of the Committees on Soil-borne Plant Pathology and Mycotoxicology, respectively of the International Society for Plant Pathology and are involved in the organization of the next meeting in 1988 in Tokyo. Financial support from The Potato Marketing Board, the Health and Safety Executive, the Overseas Development Administration and the Ministry of Agriculture, Fisheries and Food is gratefully acknowledged.

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Entomology Department

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