ROTHAMSTED RESEARCH

Rothamsted Experimental Station Report for 1984

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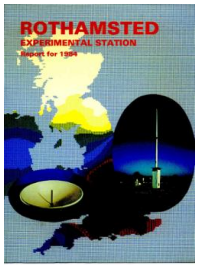

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INTRODUCTION

This report covers the first full year of the Crop Protection Division, and already the benefits of group planning, collaborative work, and shared resources are apparent. Seven major projects, each involving staff from two or three departments, are now well established, in addition to contributions from all departments to the several multidisciplinary experiments reported in the Multidisciplinary Agronomy section.

Unfortunately this progress has been tempered and hindered by staff cuts, announced in September, necessitated by the withdrawal of funds by DES, and to a lesser extent MAFF. Consequently, this Division will have lost, through normal or premature voluntary retirement, compulsory redundancy or transfer to other AFRC Institutes, rather more than 50 staff over the period 1 April 1984 to 31 March 1987, spread fairly evenly over all the component departments. Such a severe rundown of research and support expertise has meant that a disproportionate amount of time has been spent on restructuring programmes, seeking alternative funds, and not least, trying to alleviate the many personal difficulties created.

As a result of the allocation of areas of responsibility within the Agricultural Food and Research Service between the different arable institutes, the remit of Rothamsted Crop Protection Division now covers studies on the biology, ecology, behaviour and dispersal of invertebrates, fungi, bacteria and viruses, and the processes, compounds and organisms to monitor, protect and control pests and diseases of crops. Although the retrenchment will inevitably mean the curtailment or cessation of some projects, there are nevertheless major programmes unlikely to be affected, including pest and disease monitoring and forecasting, integrated control, physicochemical properties of insecticides related to translocation, structure/activity relationships of insecticides, the biology, physiology and control of nematodes on arable crops, nematode characterization, the biology, epidemiology and control of fungal and virus diseases of arable crops, aerobiology, and biodeterioration.

It is pertinent to stress also that the Division, which has excellent relationships with farmers through the 'Friends of Rothamsted', remains the largest and most experienced group of specialists working on aspects of crop protection in the Service. It retains enormous potential and will continue to produce useful fundamental and applied studies relevant to crop protection and environmental conservation.

ENTOMOLOGY DEPARTMENT

For many years much of the Department's work has been aimed at minimizing pesticide use through improved monitoring and forecasting methods, modification of cultural practices, basic studies on the behaviour of pests and beneficial organisms, and the use of environmentally harmless formulations. Against the recent background of public concern

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for the environment it is worth pointing out that many leads, begun at Rothamsted years before the public clamour began, are now opening up new opportunities for providing environmentally safe and economically viable pest control, ways of monitoring environmental change, and of reducing contamination from various agricultural and industrial practices. This report draws particular attention to studies relevant to these topics within the wider Departmental programme.

Minimizing pesticide usage

Aphid surveying. The current information from the suction trap network used to help formulate advice on the need for pesticide applications was again issued weekly to the agricultural industry in the form of the *Aphid Bulletin* and *Aphid Commentary* (*Rothamsted Report for 1982,* 89). New ways of publicizing this information and making it available rapidly to farmers, for example on viewdata systems, were explored.

Progress has been made towards defining crops at risk from aphid infestation and to presenting these data in map form. Previously it had been shown that if five or more *Aphis fabae* were recorded in samples from a single suction trap by mid-June, spring-sown field beans in that area would probably require an aphicide treatment (Way *et al. Annals of Applied Biology* (1981) **98,** 21–34). By extrapolation of current suction trapping data, using the SURFACE II mapping program, the areas in which spring-sown field beans were at risk from *A. fabae* damage in 1984 were identified. ADAS reports indicate that spray thresholds were exceeded in some fields in the areas thought to be at risk, but there were also some fields in the South West and West Midlands in which unexpected outbreaks occurred.

The accuracy of aphid forecasts will improve when the factors that cause variations between fields in the same region can be identified. For cereal aphids the abundance of natural enemies has been identified in a simulation model and a recently developed decision tree, as an important factor affecting population development in some years (Dewar and Carter *Bulletin of Entomological Research* (1984) **74,** 387–398). Therefore, in 1984, the Rothamsted Insect Survey Cereal Aphid Monitoring Scheme (RISCAMS) (*Rothamsted Report for 1982,* Part 1, 92–93 and *Rothamsted Report for 1983,* 89–90) was modified to indicate aphid density and natural enemy abundance and activity in 20 winter wheat fields near Rothamsted.

Because of the dry autumn of 1983 many commercial winter wheat crops were sown early and became infested by immigrant aphids after emergence. These aphids overwintered successfully, as in 1981–82 and 1982–83. Some were found in crops in late May, but numbers remained low until mid-June when a large immigration of *Sitobion avenae,* monitored by the Rothamsted suction trap, persisted from ear emergence to flowering (Zadoks growth stage 51–69). This led to a rapid increase in aphid populations in 19 fields, which were sprayed after flowering in late June to early July when populations were generally greater than ten aphids per tiller and still increasing. Recolonization of these fields by alate aphids occurred soon after spraying, but a further application of aphicide was unnecessry. The only unsprayed field was of Rapier, a semi-resistant cultivar, sown late (22 October 1983). Except for this field, the variation in aphid populations between fields was small although high densities were reached slightly sooner in early-sown fields. The effects of natural enemies on aphid populations were small in 1984, although preliminary results suggest there were differences in the numbers of natural enemies between fields. The failure of natural enemies to control aphid populations in 1984 was simulated by a mathematical model run using maximum and minimum temperatures and suction trap data from Rothamsted, but with the effects of natural enemies excluded. Its predictions were close to observed densities up to the time

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of spraying. Work to refine simulation modelling as an approach to predicting the need for, and timing of pesticide applications will continue. (Carter, Tatchell, L. R. Taylor, Woiwod; Dupuch, French, S. J. Parker, Riley, Rowe, M. S. Taylor and Wood)

Elemental analysis of insects. Studies on the elemental composition of aphids were continued to try to determine whether different populations have their own natural markers in the form of characteristic combinations and concentrations of elements (chemoprints), and whether the chemoprints of individuals from virus-infected and virus-free plants are distinguishable.

Further work with two species of cereal aphid, the rose-grain aphid *(Metopolophium dirhodum)* and the bird-cherry aphid *(Rhopalosiphum padi)* confirmed earlier preliminary results: the winged and wingless forms, of both species, have different chemoprints, and the winged aphids have a more characteristic chemoprint than the wingless ones, at least in the bird-cherry aphid.

The elemental composition of the wingless aphids is generally uniform possibly because clones of parthenogenetically reproducing species, such as those examined here, are genetically uniform. Nevertheless, even wingless aphids do sometimes have characteristic elemental compositions associated, apparently, with unfavourable conditions for aphid growth. For example, healthy oats are a poor host for the bird-cherry aphid, but plants infected with barley yellow dwarf virus (BYDV) are more suitable and wingless aphids from these two sources can be discriminated by their chemoprints. There is even better discrimination between winged bird-cherry aphids from healthy and BYDV infected oats, perhaps because of metabolic differences between the winged and wingless forms or because the winged aphids are genetically more variable.

Whatever the reason, the ability to differentiate, with considerable accuracy, between winged aphids from the healthy and diseased plants is of particular relevance to the study of the epidemiology of BYDV. The application of this result to field situations requires further work on the influence of different host plants from different localities, and on the proportions of aphids from diseased host-plants that are infective. (Bowden and Sherlock, with Digby, Statistics Department, and Plumb and Turner, Plant Pathology Department)

Pheromonal monitoring to time pesticide applications. One of the more successful and widely used pest monitoring schemes in the UK devised at Rothamsted, with the assistance of ADAS, is that for pea moth based on sex attractants (*Rothamsted Report for 1982,* Part 1, 97). The system has now been operated commercially for five years, and a recently completed survey has confirmed its value as an aid to rational pesticide use. The survey, covering 40 sites each year during 1980–84, has confirmed that the correct use of pheromone traps enables growers to distinguish pea crops requiring insecticidal sprays and indicates when those sprays should be applied. Application of insecticide on the predicted date (−4 to +2 days) on average greatly reduces damage compared with control plots ($P<0{\cdot}001$), whereas failure to spray at the correct time results in erratic and poor control with similar damage on sprayed and control plots ($P>0{\cdot}05$). This provides firm evidence that adequate pest monitoring can lead to improved control with less pesticide. (Wall; Garthwaite, with Dr J. Blood Smyth, ADAS Cambridge)

Following this success, work on the development of pheromonal monitoring systems for two more pests, pea midge *(Contarinia pisi)* and oilseed rape pod midge *(Dasyneura brassicae)* has gathered momentum. Much basic biology remains to be worked out, but already pilot monitoring trials have been done at five emergence sites or commercial farms, in which the information gathered from traps containing live virgin females was compared with that from non-attractive water traps and soil cores (the traditional method

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of monitoring emergence of this pest). The virgin female traps proved to be very effective indicators of emergence, having many advantages over other methods (e.g. easy to use, catches relatively easy to sort). One unusual feature of the pheromone traps was a good correlation between trap catch and population density at some sites, a feature that might enable similar traps to be used for sophisticated monitoring. A more extensive monitoring trial using more specific traps containing female extract will be done in 1985. (Wall; Garthwaite, Morris and Sturgeon, with Dr J. Blood Smyth and Miss J. McConchie, ADAS Cambridge, Mr G. Murdoch and Mr P. Hancocks, ADAS Leeds, Mr A. Biddle and Mr P. Jackson, Processors and Growers Research Organisation)

Much work was also done on the chemistry of the female sex pheromone and the design of a trap for monitoring. An electrophysiological technique for recording from single circumfila sensilla on the male antenna was perfected. These sensilla respond to the female sex pheromone and the technique is being used as an assay for active chromatographic fractions of both female extract and pheromone entrained on to carbon discs from air passed over live virgin females. The results so far indicate a multi-component pheromone. Field trapping experiments have shown that crude female extract presented on cotton dental rolls is active at a dose of three insect equivalents (IE) whereas entrained pheromone is active at a dose of 100 midge h^{-1}. Extract with an initial loading of 10 IE per trap remains active for at least five days. A number of trap designs were tested to find an efficient, specific monitoring trap. Several designs based on a small sticky cylinder performed well, and two designs that caught pea midge almost exclusively will be evaluated further in 1985. (Wall; Garthwaite, Morris and Sturgeon, with Merritt, Pickett and Wadhams, Insecticides and Fungicides Department)

Oilseed rape pod midge causes premature shattering of pods and loss of seed but control is difficult because so little is known of its biology and it is difficult to detect in the field before damage is done. As with the pea midge, field and laboratory studies have demonstrated the presence of a powerful sex attractant which might be exploitable for monitoring the pest.

Midge emergence was monitored in a field of winter wheat following winter rape attacked by midge the previous year. Larvae overwinter in cocoons in the soil and pupate in spring. The first pupae found were from soil cores collected on 8 May and more were found until 9 July. Adults were caught continuously from 18–25 May until 13–20 July, indicating an extended nine-week emergence period. Whereas emergence traps caught equal numbers of the two sexes, 99% of adults in water and sticky traps were male, because they were the more active fliers at the emergence site.

A crop of winter rape nearby was first infected by adult midge during 18–25 May and thereafter they were caught within the crop continuously until harvest. During the first month of infestation before second generation adults had emerged, 94% of adults caught were female, probably because it is mainly these that migrate from the emergence sites to rape crops. Mature midge larvae emerged from plants daily from 14 June until harvest, with two peaks of emergence clearly reflecting the two overlapping generations.

Olfactometer studies showed that males are attracted by odour from virgin females but not by odour from mated females or males, suggesting that only virgin females produce a male-attracting sex pheromone. At the emergence site traps baited with virgin females or with crude extracts of females caught many males. Identification of this female sex pheromone coupled with the design of suitable traps could lead to a monitoring system capable of warning growers of the risk of attack, enabling more effective control. (Williams; Martin, with Dr M. Kelm, Agricultural Academy of Wroclaw, Poland)

Biological control of aphids by parasites. Five years' work on the role of parasites (Aphidiidae) in helping to prevent outbreaks of cereal aphids on winter wheat provides a

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good example of the type of long-term investigation required to explore non-pesticidal control in cereals.

The presence in spring of heavily parasitized aphid populations in winter cereals is important in limiting early population growth of these pests. The aphid- and plant-derived chemicals which govern parasite behaviour, such as host-finding and reproduction, are being investigated with the aim of developing techniques for monitoring spring parasite populations and of devising novel ways of improving parasite efficiency at low aphid densities.

In preliminary trials in an olfactometer, female parasites responded to odours from their aphid hosts, whilst both male and female parasites responded to odours from the food-plants of their aphid hosts. Male parasites also responded to odours from female parasites, suggesting the existence of sex pheromones in this insect group. (Powell)

In the laboratory, crude physical models of female parasites were treated with solvents in which virgin females had been immersed. These treated models stimulated copulation attempts by the males in the laboratory. In field trials using pheromone traps baited with live virgin female parasites, large numbers of males were caught in traps containing females of the same species compared with small numbers in unbaited traps and in traps baited with females of a different species. The potential of these sex pheromones for use in monitoring parasite activity and abundance in crops, particularly in spring, is being investigated.

In another series of laboratory trials, artificial aphids were treated with crude aphid extracts and attached to young wheat plants. These stimulated oviposition attempts by female parasites. Knowledge of the stimuli which induce foraging behaviour and host recognition could be useful in developing ways of increasing parasite impact at low aphid densities. (Decker; Powell)

In addition to being attacked by parasites, cereal aphids are killed by many other natural enemies. In order to develop accurate aphid forecasting based on monitoring data it is important to understand interactions between different natural enemy groups. To this end, cereal aphids were exposed in the laboratory to adult parasites and then, after varying time intervals, to fungal pathogens (Entomophorales). Parasitized aphids which were infected by fungi within two or three days of being parasitized were killed by the fungus before the parasite could complete its development. Consequently, the impact of parasites on an aphid population in the field is likely to be reduced in the presence of high levels of pathogen infection. Work has also begun on the interactions between parasites and aphid predators. (Powell, Wilding).

Effective, non-contaminating molluscicides. Attempts have continued to find improved methods of controlling slugs without harmful side-effects on plants or vertebrates.

In feeding studies with a variety of naturally-occurring materials the rate of ingestion of agar-based test bait by the slug *Deroceras reticulatum* was increased most by sugars. Sucrose was the most potent phagostimulant on a gram-molecular basis but concentration was critical; increases over 2·5% w/v progressively reducing the volume of bait ingested. Addition of the conventional bait poisons, metaldehyde and methiocarb, progressively decreased the amount consumed, repellancy beginning at concentrations as low as 50 ppm.

Development of an effective contact-action molluscicide would reduce the risk to wildlife inherent in toxic baits, and a number of poisons which are absorbed by slugs crawling over dispersed formulations have been identified in laboratory tests. The main difficulty is in protecting toxicants from inactivation on contact with soil. Protective formulations developed with the Laboratory of the Government Chemist have shown

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improved uptake and persistence on wet soil. (Henderson; Fisher, K. A. Parker, with Briggs, Chemical Liaison Unit, Pickett, Insecticides and Fungicides Department, and Dr H. J. Prosser, Laboratory of the Government Chemist)

Differences in the susceptibility of potato cultivars to slug damage were attributed to some property localized in the tuber skin. In laboratory feeding trials removal of tuber periderm reduced but did not destroy intercultivar differences in acceptability, but removal of periderm and outer cortex made all tubers equally acceptable. In field experiments damage was reduced most by applications of methiocarb pellets, at ×3 the recommended rate, repeated at three-week intervals from July onwards. A smaller reduction in the proportion of tubers damaged was achieved by leaving some weed cover as an alternative food. Varying the time of haulm destruction did not reduce tuber damage levels but earlier lifting did. (Airey and Henderson)

Environmental monitoring by light traps. Only by maintaining long-term observations can changes, however caused, in the flora and fauna of agricultural land and conservation areas be properly monitored and verified. Rothamsted has made a unique contribution to this type of study through its light trap survey of moths. From 1933 to 1936 and again from 1946 to 1949 Dr C. B. Williams operated a light trap on the edge of the classical experiment, Barnfield, from which all macrolepidoptera were identified as part of an investigation into the effect of weather on insect flight. In 1950 a similar trap was started on the same site by L. R. Taylor to repeat the original experiment and as the first site in an investigation of insect distribution. Gradually a network of traps was established for the long-term monitoring of moth populations throughout Great Britain as part of the Rothamsted Insect Survey. The growth of the system is illustrated in the maps of trap distribution from 1964 to 1981 published in Part 2 of this *Report* and tables of annual catches of migrant and pest moth species which have been published in *Rothamsted Reports* since 1965.

The information from this survey provides a unique database on moth populations and presently contains over 1500 site-years of information for over 600 species of moths. These data have already been used extensively to develop techniques for analysing the spatial distribution of insects, in studies of diversity and land use, and have had important practical applications in many insect sampling systems. In addition to providing such information to complement the Survey's data on aphids in suction traps, the light trap network has monitored long-term changes in insect populations which can be used to investigate the environmental factors contributing to these changes.

Analyses have been done to study the effect of urbanization on moth populations and the spatial and temporal replication now available from the data has been used to standardize methods for monitoring population structure. This is an essential component of environmental monitoring being the first aspect to be affected systematically by environmental change; the populations of single species are too erratic to be used effectively as early indicators of change.

Initial analysis of the longest runs of data from Rothamsted farm indicate that large changes in total population and species composition have already occurred in response to changing agricultural practice. These changes are being analysed in detail and the analysis will then be extended to other long runs of data with the prospect of picking up future effects of environmental changes as they occur. A complementary analysis is being carried out using the Institute of Terrestrial Ecology's system of land categories to investigate the relationship between land category, population structure and the distribution and abundance of individual species. (L. R. Taylor, Woiwod; Nicklen and Riley)

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Exploitation of farm wastes

It is not only profligate use of pesticides that contributes to environmental contamination; organic wastes from livestock and industrial processes also produce unacceptable pollution. The Department's very successful programme, now in its fourth year, on using earthworms to process such waste has continued and expanded into several new areas despite curtailment of public funds. This has been achieved by attracting support from the private sector.

A major boost to the research was the receipt in March 1984 of a new prestigious Environment Risk Analysis System (ERAS) Foundation Pollution Abatement Award backed by the Confederation of British Industry and the Royal Society of Arts, and presented by the Duke of Edinburgh.

Economic surveys by the National Institute of Agricultural Engineering (NIAE) indicated a greater commercial return from worm-worked organic wastes used as horticultural composts than from worm protein as animal feed, so during the last year more emphasis has been given to compost studies. The work on the biology and ecology of candidate species of earthworms and, in particular their population dynamics, has continued, and more on-farm systems have been set up.

The growth of worms in a number of new forms of organic wastes, such as the residues from paper production, spent mushroom compost, spent brewers grains and plant residues from the horticultural industry in Guernsey, was assessed. All were found suitable either in the form of the parent waste or with some organic additive.

Considerable advances were made in the engineering of worm waste separation machinery, bed loading machinery and the design of continuous processing systems. (Edwards; Bardner, Fletcher and Lofty, with Dr J. Mullett, British Earthworm Technology Ltd and Dr J. R. Phillips, NIAE)

In laboratory and large-scale field trials, two earthworm species, *Eisenia foetida* and *Eudrilus eugeniae,* were assessed for their ability to comminute the waste from a paper mill to a soil-like compost. The raw material had 35% dry matter obtained from sedimentation and pressing of the liquid waste from the paper belts. The rate of growth and the rate of reproduction of the worms was slower in paper waste than in the animal wastes tested (*Rothamsted Report for 1981,* Part 1, 103), but could be increased considerably by the addition of nutrient sources such as animal manures. In particular *E. eugeniae* grew much faster if an artificial nitrogen source (nitrates or urea) was added. The production of the compost was rapid and proceeded equally quickly with or without the addition of animal manure.

The worm-worked product was tested extensively as a plant growth substrate. The rate of germination of lettuce, radish, tomato, cabbage, dahlia and antirrhinum plants in paper waste was similar to that of control plants grown in a commercial potting compost. Only salvia seeds failed to germinate or grew for only a few days. After the addition of fertilizer, the worm-worked waste was suitable for growing tomatoes, radishes and cabbages to a marketable size, although lettuces showed signs of aluminium toxicity. Some plants such as radishes and dahlias grew much better in a mixture of paper waste with 28% peat (v/v) than in either paper alone or in the commercial compost used as a control. Tomato, capsicum and aubergine plants grown in plastic bags containing paper waste or paper mixed with peat grew as well as in commercial 'Grobags'. The temperature of the waste would have to be raised to 50°C to kill any worms present before it could be sold as potting compost. It was found that dry heat sterilization inhibited seed germination more than steam-sterilization.

Collaborative work at Glasshouse Crops Research Institute (GCRI) has shown that worm-worked paper waste is potentially useful as a casing layer in mushroom production. Fewer mushroom initials were formed on paper waste than on a control but this could be

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an advantage because it produces a more uniform crop. (Neale and Edwards, with Mr J. W. M. Smith, GCRI)

Growth of worms in spent mushroom compost. The partially decomposed straw remaining from spent mushroom beds can create serious disposal problems because it is high in salts and low in nutrients. Worms will survive in this material but do not grow well or process it rapidly. By adding pig and cattle manure to spent mushroom compost the material was made much more acceptable to worms which were then able to work it rapidly to a friable peat-like material. Different mixtures of animal wastes and spent mushroom compost were tested in the laboratory and field; 50:50 mixtures were processed most rapidly by the worms. (Challinor and Burrows)

Plant growth trials with worm-worked wastes. The potential of worm-worked wastes as horticultural growing media continued to be investigated, with help from Oaklands Agricultural College and Efford EHS. At Rothamsted twelve different vegetables and ornamental plants were grown in worm-worked wastes blended with peat. This identified physicochemical deficiencies which could be corrected, allowing the formulation of general purpose seeding and potting composts for commercial use. Magnesium and nitrogen deficiencies were apparent in tomatoes grown in all compost blends but these were easily remedied by addition of magnesium sulphate and 'Osmocote'® (15–12–15). Seedling emergence and quality of all crops tested were generally as good as, and often better, than that obtained with recommended and commercially available composts.

A trial using worm-worked wastes as blocking composts for planting out cabbages and cauliflowers into the field gave better results than a standard blocking medium, particularly with worm-worked pig waste and without addition of fertilizer.

Straw-based worm-worked wastes were tested as growing media for radishes, cabbages and tomatoes. These test plants all grew successfully in farmyard manure, pig waste and duck waste provided additional nitrogen was supplied to overcome its immobilization caused by organic matter decomposition.

Efford EHS supplied rooted cuttings of *Virburnum bodnantense, Pyracantha* 'Orange Glow', and *Chamaecyparis lawsoniana* 'Ellswood Gold' which were then transplanted into base medium amended with levels of worm-worked wastes at 5, 10, 25, 50 and 75%. Growth enhancement compared with commercial compost was observed even at the lower levels of addition, with the best results obtained at ratios of 25 to 50% v/v worm-worked waste to commercial compost. (Burrows, Edwards; Fletcher and Fraser, with Miss M. Scott, Efford EHS)

Earthworms in the assessment of materials for landfill. Work began in June 1984 on the use of earthworms as laboratory bioassay organisms for assessing the toxicity and mobility of heavy metal contaminants present in soils, sediments and sludges. This work forms part of a wider research programme sponsored by the US Government aimed at assessing the suitability of material dredged from rivers and estuaries for landfill, and follows work on a plant bioassay developed in Holland and the UK. A suitable bioassay method has been worked out in collaboration with Waterways Experiment Station, USA and the work at Rothamsted has begun by comparing the uptake of heavy metals by *Eisenia foetida* with that by common soil-inhabiting earthworms in order to assess the suitability of using *E. foetida* as a surrogate species. Soils and sediments containing high levels of at least one of the elements Zn, Cu, Pb and Cd have been collected from sites in Wales, Somerset and Holland for bioassay. The species *Lumbricus terrestris, Allolobophora longa, A. chlorotica, A. caliginosa* and *Octolasion cyaneum* have been selected for comparison with *E. foetida,* and laboratory populations of each of these species have been built up with the aim of culturing them continuously. (Stafford and Edwards)

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Earthworm and microorganism interactions. During the past year the nutritional requirements of *E. foetida* have been investigated using both organic wastes and axenic systems. An autoclavable medium comprising microcrystalline cellulose, tissue paper and water was devised in which worms could be kept alive for up to 20 weeks. The incorporation of carbenicillin into the medium rendered the worms internally and externally free of microorganisms.

The use of autoclaved media and microbially free worms allowed direct assessment of growth rates on various nutrient sources. A variety of microorganisms (bacteria, fungi and protozoa) as well as carbohydrates and proteins were tested as possible food sources. Bacteria and protozoa were found to cause rapid weight loss or death of the worms, but fungi, particularly *Arthrobotrys* species and *Cladosporium herbarum,* allowed good worm growth.

In organic wastes, there was a good correlation between worm growth and microbial populations. Worms also grew well when carbohydrates were incorporated into the waste at concentrations of 1·0% w/w or less. The addition of 1·0% glucose to cattle waste appeared to prevent the worms losing weight as the waste was well worked and they matured earlier than in unsupplemented material. All the worms died in concentrations of carbohydrates above 1·0% and in casein above 0·1%. (Morgan and Edwards, with Dr J. Beringer, Bristol University)

Honeybees

Work on honeybees has progressed along three main lines; their health, pheromonal control of colony organization and pollination studies.

Honeybee brood disease and *Varroa*. Even though this parasite has not yet been recorded in the UK work is continuing, in collaboration with European colleagues, to provide further insight into the complex association between the mite *Varroa jacobsoni* and acute paralysis virus (APV) infection of honeybees.

In Germany, in late summer, outbreaks of brood disease were observed in colonies supporting a high mite population. Samples of brood from such colonies were tested individually against acute paralysis virus antiserum by immunodiffusion. Almost all the larvae from unsealed cells gave positive serological reactions, whereas only half or less of the prepupae and pupae from mite infested sealed cells contained sufficient APV to be detected in this way.

During work at The Tierhygienisches Institut, Freiburg, live mites were collected from sealed brood cells and transferred to healthy pupae in individual tubes. One to five mites were confined with pupae at 30°C for three days; 40% of pupae confined with only one mite failed to develop and gave positive reactions to APV antiserum. This is the first direct evidence that individual field collected mites are capable of transmitting APV. However, it does not explain the high percentage of overt APV infection found in larvae from unsealed cells because *Varroa* mites do not usually enter brood cells until shortly before they are sealed. This suggests that larvae are ingesting virus secreted into the brood food by the adult bees that tend them. Previous work indicates that APV spreads as an inapparent infection in this way, via salivary gland secretions. Thus, adult bees in which APV is multiplying sufficiently to cause mortality may not only provide a source of virus which the mites can transmit to brood, but may also contribute directly to the infection of young larvae. (Ball; Allen)

Pheromonal control of queen production. When the supply of queen pheromone in a honeybee colony becomes inadequate, because the queen has died or is missing, workers

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transform some worker cells containing young female larvae into queen cells, and rear the occupants as replacement queens.

Recent work has demonstrated that the presence of either open queen cells (containing queen larvae) or closed queen cells (containing queen pupae) in queenless colonies prevents further queens being reared. This suggests that the immature queens produce inhibitory pheromone which the workers distribute throughout the colony in transferred food and by antennal contact. Such a feed-back mechanism would explain why a colony that loses its queen rears only a limited number of potential replacements.

It has also been demonstrated for the first time that the construction by colonies of queen cell cups, which are the empty cup-shaped precursors of queen cells, is related to queen pheromone deficiency and appears to be at least in part inhibited by pheromones from mature and immature queens, the former being the more effective. Variation in the amount of queen pheromone in a colony, in association with the recurring cycle of construction and destruction of queen cells, could account for variation in the number of queen cell cups present.

It has been confirmed that a mature queen stimulates foraging for both nectar and pollen, probably by a pheromone, and that immature queens also stimulate foraging. Whereas immature queens are less effective in stimulating nectar collection than mated laying queens, they are more effective in stimulating pollen collection.

If the pheromone produced by the immature stages of queens was isolated and synthesized it might be valuable for encouraging pollination of crops on which pollen-gatherers are the most successful pollinators, and for inhibiting queen rearing in colonies with a mated laying queen. (Free; Ferguson and Simpkins)

Pollination of oilseed rape. It may eventually be possible to extend the study of foraging stimulants described above to improve the pollination of field crops. One of these, oilseed rape, generally yields well without insect pollination although honeybees have been reported to increase yields. Present studies are aimed at determining the relative importance of wind and insects in pollinating the cvs currently grown.

A comparison of the pollination requirements of four cvs of winter rape (Jet Neuf, Rafal, S101/79 and Lingot) and one of spring rape (Willi) grown in a glasshouse has shown that all are self-fertile, yielding equally well whether self- or cross-pollinated. However, hand-pollinated plants yield more than auto-pollinated plants indicating that insufficient pollen transfer occurs in the still air of a glasshouse. Plants that are shaken to simulate movement by wind yield better than auto-pollinated plants and as well as hand-pollinated ones. This suggests that under field conditions movement of plants by wind probably increases self-pollination of cvs that auto-pollinate poorly. Hand-pollination and the shaking of plants increases the set of early flowers. Further studies are being made to determine whether additional pollination by insects in the field is of economic benefit, by producing shorter plants with evenly ripening pods which would be easier to harvest.

Samples of the air taken over a crop of flowering rape (cv. Jet Neuf) with an automatic volumetric spore trap, revealed large quantities of airborne rape pollen, particularly on warm, dry, sunny days. This pollen may be carried to the stigmas of older flowers in which the stigmas protrude above the corollae, so wind is probably an important pollinating agent, causing not only self-pollination of rape flowers by moving them but also cross-pollination by transporting pollen through the crop. (Williams; Hadley, Martin and Tomkins)

Staff and visiting workers

During the year J. Bowden and L. R. Taylor retired; it is a pleasure to acknowledge their distinguished contribution over many years to the Department and to their subject.

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Twenty-one staff participated in a wide range of international and UK society meetings and working visits. Particularly notable were C. A. Edwards' organization of a large international conference on 'Earthworms in Waste Management and Environmental Improvement' at Cambridge and his plenary address to the Jubilee Symposium of the Zoological Society of South Africa, L. R. Taylor's lecture tour to several universities in the USA, and J. Bowden's attendance at a seminar on the 'Use of Traps in Vector Research and Control' in India. The Department was well represented, providing many papers, at the British Crop Protection Conference, Brighton, the 9th LARS Symposium on 'Rational Pesticide Use', the Association of Applied Biologists' Symposium on 'Crop Protection in Evolving Agriculture', and at several IOBC and apicultural meetings. Brenda Ball spent three weeks on a working visit on Varroasis to the Tierhygienische Institut, Freiburg and R. Bardner a similar time with the Coffee Rehabilitation Programme in Uganda.

K. Allen, Theresa Minall, Amanda Modlen and Nicola Rowe resigned; N. Carter, Elizabeth Stafford, E. R. L. Fisher, Veronica French and Linda Muir were appointed. Nine students or visiting workers joined the Department for varying periods—R. ffrench-Constant, Clare Lanchbery, Jacqueline Penny, T. Harper, Alison Challinor, Maria Kelm, Neonili Sitaropoulou, L. Allen-Williams and Jesusa Cristosomo. G. M. Tatchell and J. E. Ashby were promoted and A. M. Dewar, M. Russell, Fiona Fraser and Marjorie Bellingham transferred to other departments. I. Burrows and M. Cornwell remained as visiting staff supported by British Earthworm Technology Ltd.

INSECTICIDES AND FUNGICIDES DEPARTMENT AND CHEMICAL LIAISON UNIT

The work of the Department and the Unit has been rationalized into six major groups covering structure/activity relationships in synthetic insecticides derived from natural products, behaviour controlling chemicals from natural products, electrostatically charged spray application systems, resistance in insects, resistance to and efficacy of fungicides, and the influence of physical properties on pesticide behaviour. By this rationalization and by establishing closer collaboration with other groups in the Division and in Universities, the impetus of work will be maintained, despite the impending staff losses.

The new studies on physical properties will aid the discovery of new pesticides at Rothamsted and elsewhere. This input is particularly useful for modifying the properties of insecticides developed from natural products such as the natural pyrethrins, and also for predicting the environmental behaviour of all pesticides. Natural products are being exploited in two ways leading to (i) safer and very effective insecticides that can be used at very low rates, and (ii) selective agents which affect the behaviour of target species. Further improvements in reducing environmental contamination can be achieved with improved spray application systems which direct more of the active chemical on to the target area of the crop. A constant concern is that pests are developing resistance, which often leads to increased applications of pesticides which eventually become ineffective. Much of our research on the biology of insecticide and fungicide use is directed to avoiding these problems by developing new strategies for use.

The outstanding contribution to the study of structure-activity relationships in lipophilic insecticides made by Dr Michael Elliott, who retires this year, has had tremendous impact on agriculture and the agricultural chemicals industry, particularly the discovery of safe and highly effective pyrethroids such as deltamethrin. This work will be actively continued by his colleagues under the leadership of Dr Norman Janes. Their most recent work, some of which is reported here, encompasses two new and exciting developments.

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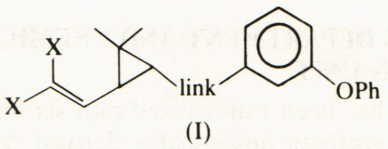
Relationships between molecular structure and insecticidal activity

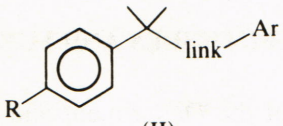
New agents to control insect pests continue to be essential for soundly-based management programmes, and natural products (for example, the pyrethrins) have been the basis from which the Department has, in the past, established principles governing the dependence of various useful types of activity on structure. Advances in two new areas can now be reported following deferment of publication during patent procedures. One development arises in a fundamental departure from the structural features long considered necessary for activity in pyrethroids and the other in a chemical class, substituted amides of long chain acids, where hitherto no practical insect control agents have been discovered.

Both classes of compounds are sufficiently novel to merit examination over a larger range of insect species, including those with strong resistance, and their selectivity in the laboratory and in the field is being examined intensively.

Synthetic pyrethroids with a non-ester central group. It is now well established that each part of the pyrethroid molecule can be replaced by an isosteric group without loss of insecticidal activity; however, the potency of compounds with the central ester linkage modified is particularly sensitive to the nature of the other groups present, as well as to that of the replacing group. Consequently many early attempts to replace the $CO.OCH_2$ link in benzyl chrysanthemates (I) did not give active structures.



(I)



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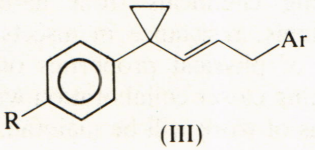

(III)

The recent significant advance has been the discovery that when the unit $-CO.O-$ is replaced by a *trans* double bond (link then $=-CH\overset{E}{=}CH.CH_2-$) some activity is retained. Specific examination of this modification has only been possible by development of a good synthetic route to the required structures.

When a *trans* ethylene is combined with a concept examined earlier (II; link=$CO.O.CH_2$) to give (II; link=$-CH\overset{E}{=}CH.CH_2$) much greater activities are developed. An alternative unit (II; link=CH_2OCH_2) is present in the related compound MTI 500 (first announced at Fifth International Congress of Pesticide Chemistry, Kyoto, 1982, and Udagawa *et al.* (1985) in *Recent Advances in the Chemistry of Insect Control,* RSC Special Publication No. 53, p. 192).

Detailed examination of the possible variations in this new type of structure has led to the following conclusions:

1. Reverting to link=$COOCH_2$ in II destroys activity, as does replacement by *cis* $-CH\overset{Z}{=}CH.CH_2$, or $C\equiv C.CH_2$.
2. The gemidimethyl group can be replaced, generally advantageously, by cyclopropyl, e.g. (III, Ar=3-phenoxyphenyl).
3. When Ar is 3-phenoxyphenyl, the compounds are usually more active than with other pyrethroidal alcohol residues. 4-Fluoro substitution in this group enhances activity, as it does in conventional pyrethroids.

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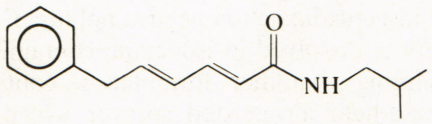
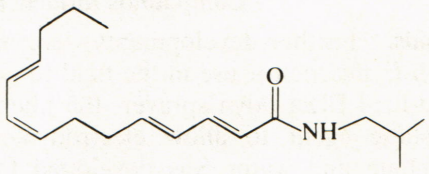
4. Activity is sensitive to the nature and position of the R substituent.
5. In an especially active compound (III, R=4-EtO, Ar=4-fluoro-3-phenoxyphenyl), hydrogenation to the corresponding alkane (link=$CH_2CH_2CH_2$) lowers activity to two test insect species. c.f. MTI-800: (II, R=4-EtO, link=$CH_2CH_2CH_2$, Ar=4-fluoro-3-phenoxyphenyl).

Insecticidal Amides. Insecticidal activity has been known in long chain aliphatic isobutylamides for many years. However, of 159 naturally occurring amides, related to those found predominantly in Piperaceae species, only 28 have been shown to have even a trace of insecticidal activity, and no structure/activity relationships were obvious from the available information. Major difficulties were the instability, and multi-isomer mixtures, encountered with these typically polyenic amides.



(IV)



(V)

A first important step was the discovery of a stable, well characterized compound (IV), analogous to tetrahydroanacyclin (V) and the replacement of diene by phenyl (an approach which had succeeded well in the pyrethroid field). IV had significant activity against both houseflies and mustard beetles, but even this diene rearranged readily under the conditions of synthesis. Monitoring and chromatographic separation were necessary to identify the insecticidal compound, in the presence of inactive isomers.

Adopting a structure/activity investigation strategy similar to that used in pyrethroids, extra groups were introduced into the molecule to identify regions where variation was possible. This led to the following conclusions:

1. Increase in the size of the N-alkyl group by one or two carbon atoms is possible, and even raises activity in some cases, as long as the isobutyl structure is present.
2. Substitution by methyl in any of the five positions of the pentadienyl chain lowers activity severely.
3. The influence of substitution on the ring depends on the nature, number and position of substituents. While no correlation is immediately apparent this identifies an area where optimization is possible.
4. Changing the phenyl group to other aromatic systems has unpredictable effects. One particularly promising alternative aromatic system is β-naphthyl, which is comparably amenable to the effects of substituents.
5. Other changes, such as replacement of CH_2 by O, and extending the saturated chain length (from (CH_2), up to as much as $(CH_2)_{12}$) generally diminish activity, but a secondary peak in activity has been observed in some compounds.

Insecticidal selectivity. Screening of established and recently developed synthetic pyrethroids has continued using *Chrysoperla carnea* larvae, and has been extended to *Myzus persicae* adults. *C. carnea* is of considerable interest because of its natural tolerance to many insecticides and in particular its ability, demonstrated by Ishaaya and Casida (Environmental Entomology (1981) **10,** 681–684), to metabolize *cis* esters. *M. persicae* is included because of availability of resistant strains, and the current interest in pyrethroids for aphid control.

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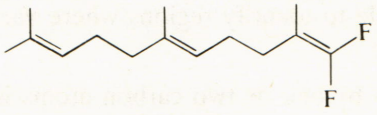
Tests with established pyrethroids provide the following conclusions: *Trans* esters (e.g. bioresmethrin) are more active against *C. carnea* than *cis* (e.g. cismethrin, cypermethrin, deltamethrin) in contrast to many other species. Most significantly a cypermethrin *cis* isomer (WL 85871) is less toxic than cypermethrin itself, with *trans* isomers present as well, supporting Ishaaya and Casida's observations. *M. persicae* conforms to a more normal pattern, i.e. toxicity decreases in the order deltamethrin, cypermethrin, permethrin, bioresmethrin.

This work is currently being extended to the new compounds described immediately above, and to include assays with resistant *M. persicae*. (Chemical work: Elliott, Janes; Baydar, Johnson, Khambay and Pulman. Biological work: Farnham, Stevenson; Adams, Robertson, Smart and Walters)

Compounds influencing behaviour of invertebrates

Aphids. Further developments were made in formulating the aphid alarm pheromone (*E*)-β-farnesene for use in the field to improve insecticidal action against aphids. For the hand-held Electrodyn sprayer, the pheromone was dissolved in isooctane containing an antistatic agent to allow electrodynamic charging. Another formulation containing isooctane and water was developed for a hand-held air-assisted sprayer which used deflectors and air to project a narrow swath of fine charged drops. This equipment was used to apply pheromone to seed potato plants infested with *Macrosiphum euphorbiae* and significantly increased the effectiveness of demeton-S-methyl against aphids on the middle leaves, thus confirming results from last year (*Rothamsted Report for 1983,* 100) that this method is particularly suitable for enhancing insecticide control of *M. euphorbiae*.



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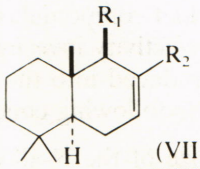
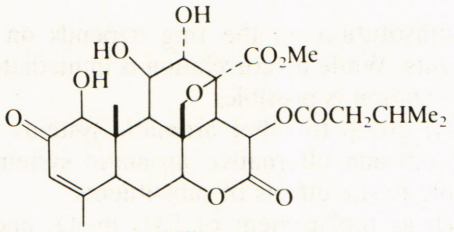


(VII) R_1, R_2=CHO
(VIII) R_1=CO_2Me
R_2=CO_2H



(IX)

Extension of studies on pheromone application techniques requires a pheromone analogue that can be more easily detected than the pheromone itself. Further investigation of fluoro analogues of (*E*)-β-farnesene has resulted in a new compound, 1,1-difluoro-2-6,10-trimethylundeca-1,5,9-triene (I), which is highly active, easier to prepare and more stable than previous fluoro analogues (*Rothamsted Report for 1983,* 100).

(−)-β-Caryophyllene, the aphid alarm pheromone inhibitor, was found in large amounts not only in hops but also other crop plants, e.g. potato, carrot, and cotton. Although most plants contain some (*E*)-β-farnesene, (−)-β-caryophyllene is always

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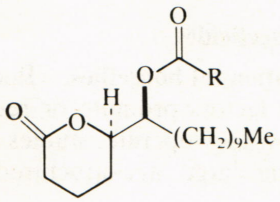
present at a greater concentration. The headspace vapour above some of these plants inhibited alarm response. In an atmosphere of (−)-β-carophyllene *Myzus persicae* showed diminished response to synthetic (*E*)-β-farnesene, and to natural pheromone released from aphids during attack by a predator *(Chrysopa carnea)*.

(−)-Polygodial (VII) is a potent aphid antifeedant, but the synthetic (±)-polygodial is phytotoxic. This has now been resolved into the component optical isomers by separating diastereomeric salts formed between the racemic mono-acid precursor (VIII) and optically pure α-methylbenzylamine. The (+)-isomer is highly phytotoxic alone, and traces contained in the isolated (−)-isomer cause leaf damage. However, production of natural (−)-polygodial on a large scale is now possible by cultivation of the parent plant, marsh pepper *(Polygonum hydropiper),* from seed.

Other antifeedants have been tested against aphids, including a series of quassinoids. The most active is isobruceine A (IX) which, at a concentration of 0·01%, deters colonization of Chinese cabbage *(Brassica pekinensis)* leaves by aphids.

Mosquitoes. The absolute configuration of the mosquito *(Culex pipiens fatigans)* oviposition attractant pheromone (*Rothamsted Report for 1981,* Part 1, 132) is (−)-(5*R*,6*S*)-6-acetoxy-5-hexadecanolide (Xa). Identification was by chromatography of the 6-trifluoroacetoxy derivatives (e.g. Xb) of the natural pheromone and of the synthetic (−)-(5*R*,6*S*)- and (+)-(5*S*,6*R*)-enantiomers on a capillary column having a chiral stationary phase comprising a derivative of (1*S*,3*S*)-chrysanthemic acid (XI). The synthetic (−)-(5*R*,6*S*)-enantiomer (Va) stimulated the mosquito to lay four times as



(X) a: R=CH_3
b: R=CF_3

(XI)

many egg rafts as in the control ($P<0·01$) whereas for the (+)-(5*S*,6*R*)-enantiomer there was no statistically significant attraction. The racemic trifluoroacetoxy derivative was itself a highly active oviposition attractant and at a level equivalent to 10 egg rafts (i.e. 3 μg pheromone) attracted oviposition of 290 egg rafts against 31 for the control ($P<0·001$) in 10 replicates. (With Laurence, London School of Hygiene and Tropical Medicine)

Pea and bean weevil, *Sitona lineatus* (L.). Field studies with live *S. lineatus* confined on broad bean plants, *Vicia faba,* demonstrated that an aggregation pheromone is produced by male weevils. Extracts of volatiles produced by weevils feeding on beans were obtained by an air entrainment technique. Gas chromatography (GC) and coupled GC-electroantennogram analyses (GC-EAG) revealed the presence of a number of electrophysiologically-active compounds, one of which was associated only with male weevils. This substance was identified (by GC-mass spectrometry) as 4-methyl-3,5-heptanedione (XII). GC-MS, GC and GC-EAG data of an authentic synthetic sample of XII were identical with those of the weevil-produced substance. Bean volatiles stimulating the antennae of *S. lineatus* were identified (by GC-MS and GC-coinjection with authentic samples) as (Z)-3-hexen-l-ol (XIII), (Z)-3-hexen-1-yl acetate (XIV) and linalool (XV).

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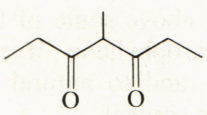
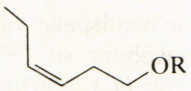
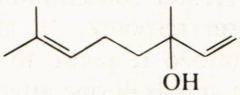


(XII)



(XIII) R=H
(XIV) R=$COCH_3$



(XV)

Confirmation that XII is an aggregation pheromone was obtained from a factorial field experiment in which weevil responses to substances XII–XV were studied. XII attracted both sexes ($P<0{\cdot}001$) and in some situations the response was synergized by a mixture of XIII–XV ($P=0{\cdot}05$).

In the spring, the combination of XII–XV is very efficient in attracting weevils into cone traps from overwintering sites. Synthetic aggregation pheromone could therefore be used to detect or monitor population levels of the insect.

Slugs. Experimental seed treatments using methiocarb, cartap and thiocyclam are effective against slug damage in winter wheat but are too toxic to vertebrates to warrant development. In laboratory tests using *Deroceras reticulatum* a series of crude plant extracts and components, including geraniol, were shown to prevent feeding on wheat seeds. One plant extract was also very toxic to slugs, causing curling of the mantle, a symptom not reported before, and copious mucus secretion.

(Pickett; Blight, Briggs, Cayley, Dawson, Greenway, Griffiths, Mudd, Scott, M. C. Smith, Wadhams, H. S. Williams, Woodcock)

Resistance to insecticides

Selection of insecticide resistance in caged populations of houseflies. Because of technical difficulties in determining unequivocally which factors promote or retard the development of insecticide resistance in the field and how they operate, studies were made under controlled conditions in the laboratory, using large age-structured populations of houseflies.

Using a strain of houseflies with proven potential to resist pyrethroids, panels with residues from the highest rates of permethrin initially gave good control, but quickly failed because *kdr* was selected rapidly. Lower rates selected for resistance less rapidly but gave poorer initial control. This supports field observations in implying that the use of residually applied persistent pyrethroids against populations of houseflies in 'closed' buildings on animal farms in the UK is ultimately self-defeating, since application rates giving adequate control quickly select for strong resistance.

The evaluation of other options for control of flies with insecticides under these conditions has been started.

Characterizing insecticide-insensitive acetylcholinesterase in different strains of houseflies. Inhibition kinetics measured with the aid of a microcomputer interfaced to a spectrophotometer (*Rothamsted Report for 1983,* 104) have identified in field and reference strains from other laboratories four homozygous forms of acetylcholinesterase, each with a different spectrum of response to the inhibitors. A further two were heterozygous, one of the components being much more sensitive to inhibition than the reference susceptible enzyme. This polymorphism illustrates the adaptability of the gene for this enzyme, the target for two major classes of insecticides.

Rapid identification of insecticide-resistant *Myzus persicae*. Antiserum to carboxylesterase E4 (*Rothamsted Report for 1983,* 104) was used to measure the amount of this

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enzyme in individual aphids, as a means of diagnosing their resistance status. By eliminating the contribution of non-specific esterases, slightly (R_1) and very (R_2) resistant individuals were distinguished faster and more simply than by total esterase assay or electrophoresis.

Genetic basis of insecticide-resistance in *Myzus persicae*. Previous work had indicated that the larger amounts of E4 in resistant aphids might arise by gene amplification, so the molecular biology of this resistance is being investigated. RNA was extracted from susceptible (S), and very resistant *M. persicae* clones, the poly $(A)^+$ fraction purified by affinity chromatography and sucrose gradient centrifugation, and analysed by translation with proteins using rabbit reticular lysate. There were no gross differences between the mRNA from the two sources when total translation products were characterized by SDS electrophoresis. However immunoprecipitation with E4-IgG revealed much larger amounts of a particular protein in the incubations with mRNA from resistant aphids. The slightly faster (lower mol. wt.) electrophoretic mobility of this protein than the E4 reference suggested post-translational modification of the native protein. This was confirmed and identified as glycosylation, by lectin affinity chromatography and sugar-specific staining of electrophoretic gels. The presence of larger amounts of mRNA for the nascent E4 in resistant aphids is consistent with the hypothesis of gene amplification, i.e. multiple copies of the E4 structural gene.

Effects of insecticides on resistant *Myzus persicae*

Deltamethrin. Treatment of crops with deltamethrin should help prevent virus transmission by *M. persicae* if its rapid knockdown action (*Rothamsted Report for 1983*, 104) persists long enough to give sustained protection. Laboratory tests demonstrated that continuous exposure of S, R_1 and R_2 variants to 0·001% a.i. deltamethrin sprayed on sugar beet plants eight and 16 days previously, produced good (75% for S) and satisfactory (50% for R_1 and R_2) knockdown. Even brief (30 s) exposure caused knockdown of R_1 aphids (50% after eight days). However the application of deltamethrin has drawbacks: clean-up is poor, and it may cause resurgence. (On glass at 0·0001% a.i. deltamethrin stimulated *c.* five-fold larviposition and caused embryo expulsion in S aphids, and did likewise to R_2's at 0·001% a.i.). These drawbacks may be overcome by applying deltamethrin in combination with systemic organophosphates or carbamates. Spraying aphid-infested plants with a mixture of deltamethrin (0·001% a.i.) and heptenophos (0·04% a.i.) gave significantly better kill of susceptible, but not R_1 or R_2, aphids than either treatment alone. Deltamethrin with pirimicarb (0·03% a.i.) performed better against all classes reflecting the greater effectiveness of pirimicarb.

Aldicarb. Soil-applied aldicarb killed S, R_1 and R_2 aphids for the first three to four weeks after application, but thereafter protection was rapidly lost, survival being $R_2 > R_1 > S$. Resistance therefore allowed earlier colonization and aphid buildup, thereby reducing virus control. However many of the aphids which survived exposure to treated, BYV-infected, sugar beet were unsettled and failed to transmit this virus to indicator seedlings.

Identification of BYV in leaf discs by ELISA. The serological test ELISA detected BYV in leaf discs inoculated by *M. persicae* after excision from beet plants and incubation for seven days on capillary matting flushed with nutrient medium. This technique provides an alternative to indicator seedlings, and is particularly suited for use in determining the ability of susceptible and resistant aphids to spread virus through insecticide treated crops.

(Sawicki; Dand, Denholm, Devonshire, Farnham, Gooding, Jusseaume, Moores, Rice, Searle, Stribley, White)

https://doi.org/10.23637/ERADOC-1-25 *pp 20*

Fungicides

Fungicide research is now concentrated in two areas. September sowing of wheat has encouraged early development of take-all, against which no effective chemical control measures exist. Resistance to fungicides is a second major problem. In particular, in eyespot of cereals, resistance to methyl benzimidazole carbamate (MBC)-generating fungicides is widespread, and equally good alternative fungicides are not available. Decreases in sensitivity of barley powdery mildew to some fungicides, together with a decline in their performance on certain varieties, continues to cause concern.

Fungicides and take-all. Nuarimol (8% w.p.) was applied at 1·1 kg ha^{-1} as a water drench, and incorporated by spike rotovator into a winter wheat seedbed, on a farm on Essex boulder clay. Take-all developed late and remained slight. Nuarimol had insignificant effect until the final sample (Zadoks growth stage 77; 9 July) when it reduced the take-all rating from 182 to 117 and percentage roots infected from 44 to 20. This is comparable with effects previously seen at Rothamsted. (With J. Finnis, West Essex Farmers Agronomy Ltd.)

'Baytan' seed treatment (25% triadimenol+3% fuberidazole) hardly affected take-all in early (8 September) or later (7 October) sown winter wheat which was severely infected. However, treatment increased yield of earlier sown plots.

Work continued on the new field method for testing possible take-all control agents (see Plant Pathology Department report p. 128) using soil-applied nuarimol, the best treatment in the previous year's experiments, was used as the standard.

Fungicide resistance in eyespot. A preparatory site for a long-term study of development of fungicide resistance in eyespot *(Pseudocercosporella herpotrichoides)* was drilled with a third consecutive wheat crop. The level of MBC resistance (1·5% of lesions) was similar to that found at other Rothamsted sites in 1983, and probably represents the background level of resistance before selection pressure is applied by fungicide use, in contrast to some commercial sites outside Rothamsted where 100% of lesions yielded resistant isolates. Isolates differing in both sensitivity to MBC and in morphology were satisfactorily recovered following inoculation of field plots elsewhere.

Negatively-correlated cross-resistance provides a possible means of controlling the spread of resistance through use of suitable mixtures. In culture, and on wheat seedlings in pots, eyespot isolates resistant to MBC were sensitive to methyl N-(3,5-dichlorophenyl)-carbamate (MDPC) and *vice versa.*

The MBC resistance of eight field isolates remained stable through ten consecutive subcultures, on fungicide-free media. Seven MBC-sensitive isolates were subcultured on media with increasing concentrations of MBC. Six declined in sensitivity, but the seventh (which was unusually virulent in wheat seedlings) failed to grow even at the lowest fungicide concentration. These results indicate that resistance in field populations results from selection of already resistant isolates, rather than adaptation of otherwise sensitive strains. One isolate which initially failed to grow on medium containing 0·5 μg ml^{-1} prochloraz (an alternative fungicide for eyespot control), grew at 1·0 μg ml^{-1}, albeit slowly, after successive transfers to media with increasing levels of this fungicide. (With Creighton and Fitt, Plant Pathology, and BASF, UK)

Nature of fungicide resistance in barley powdery mildew. Mildew control in barley is largely dependent on fungicides that inhibit sterol biosynthesis. Many inhibit the C-14 demethylation step (DMI's), and isolates resistant to one of these fungicides (triadimenol) have again been encountered in mildew populations collected during 1984.

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These isolates were cross-resistant to all other DMI fungicides tested, but differences in levels of cross-resistance suggest that more than one resistance mechanism may operate. In competition experiments on both detached leaves and whole plants, triadimenol resistant isolates lacked fitness, and were soon lost from mixtures with a triadimenol sensitive isolate. No isolates were cross-resistant to morpholine fungicides (tridemorph, fenpropimorph), which act at a different step in sterol biosynthesis.

Progeny from two mildew crosses showed a continuous distribution in triadimenol sensitivity, indicating that resistance is controlled not by one gene, but probably by many. A cross between two triadimenol-sensitive isolates yielded progeny less sensitive to the fungicide than either parent, suggesting that triadimenol sensitivity may arise through different gene combinations. Recombination failed to produce progeny more resistant to triadimenol than any encountered in field populations. Mutagenesis using N-methyl-N′-nitro-N-nitrosoguanidine failed to generate resistant isolates, although mutants with altered virulence characteristics were produced. Mutants either lost virulence characteristics, or gained additional ones.

Resistance to fungicides that inhibit sterol biosynthesis might involve changes in membrane lipids. To examine this possibility, we first characterized the lipids in several mildews. Ergosterol was completely absent from four powdery mildews (apple, barley, cucumber, pea). Instead $\Delta^{5,28(28)}$ ergostadienol was the major sterol, together with smaller amounts of the $\Delta^{7,24(28)}$ isomer and two, as yet unidentified, sterols. The major fatty acids in barley mildew conidia were behenic (22:0) and lignoceric (24:0), but amounts were considerably reduced in membrane-enriched fractions, and both were absent from mycelia of pea mildew. Behenic and lignoceric acids, together with some unidentified longer chain fatty acids, seem to be storage lipids. Palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2) and linolenic (18:3) were the main membrane fatty acids. Qualitative changes in sterols and fatty acids were not correlated with triadimenol resistance in barley powdery mildew. In one resistant isolate the total sterol content seemed reduced, and the fatty acid levels increased. When a resistant isolate was grown on barley leaves containing this fungicide, C-14α methyl sterols were not present in the conidia, suggesting that the fungicide either failed to reach the target, or the active site was altered. (With Loeffler, LARS)

Recognition events in bean anthracnose. Specificity of races of anthracnose *Colletotrichum lindemuthianum* towards bean *(Phaseolus)* cultivars with different resistant genes is similar to that found in rusts, mildews and smuts. Yet *C. lindemuthianum* offers considerable advantages over these pathogens for studies aimed at identifying virulence gene products, since it can readily be grown in defined media. In addition, the sexual stage *(Glomerella lindemuthianum)* has recently been reported to occur under laboratory conditions. We have repeated this work with isolates imported from Brazil, and perithecia have been obtained in at least one cross. Attempts are also underway to obtain suitable fungicide-resistant and auxotrophic marker strains for use in a genetic analysis of pathogen virulence/host plant resistance. (With Bailey, LARS)

(Bateman, Hollomon; Butters, Chamberlain, Clark, Coskun, C. Smith)

Spray application

The main objects of the 1984 trials were to continue to compare the Jumbo electrostatic sprayer with other spray systems, in collaboration with sprayer manufacturers and chemical companies, and to investigate ways of increasing penetration of electrostatically charged sprays into crop canopies.

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Performance of electrostatic spray systems. When fungicides were applied for control of eyespot at Zadoks growth stage 30 in winter wheat on three sites, the Jumbo sprayer gave larger deposits on the plants than did the Spraycare ES system, or hydraulic sprayer, the mean deposits being 114, 71 and 56 $\mu g\,g^{-1}$ respectively. Charging had only little effect on deposits achieved with the Spraycare ES system but visibly decreased drift. The higher deposits achieved with the Jumbo were mainly on the leaf blades, and similar amounts of chemical were deposited by all the sprayers on the leaf bases, where eyespot infection occurs. In winds of 5–6 $m\,s^{-1}$ hydraulic and electrostatic sprayers gave deposits 2–2·5 times greater than those achieved under still air conditions. When fungicides were applied to spring barley for mildew control the increased deposits obtained by the Jumbo on the upper parts of the plant caused phytotoxic effects with the formulation used. (With Ciba-Geigy)

Penetration of charged sprays. Large deposits at the top of the plant canopy do not always lead to optimum biological effects. For example, when a mixture of mecoprop and isoproturon was applied to control weeds in winter wheat, in particular volunteer field beans, and small cleavers (hidden beneath the cereal), all treatments completely controlled field beans but cleavers were controlled better by hydraulic than electrostatic treatments because the hydraulically applied sprays penetrated better. Similarly, blackgrass in winter wheat was controlled more effectively by hydraulic sprays of isoproturon because less of the chemical applied electrostatically reached the weeds and the soil.

To overcome the failure of charged drops to penetrate deeply into crop canopies, several strategies have been investigated:

Effect of spray timing. In winter barley fungicide sprays were applied at Zadoks growth stage 21 when the crop canopy was open and therefore more suitable for electrostatic spraying. Electrostatic sprayers deposited 2–3 times more fungicide on the plants than the hydraulic sprayer and half doses applied electrostatically gave control of mildew and *Rhynchosporium* equivalent to full doses applied hydraulically.

Effect of formulations. Pesticide formulations were modified to suit the crop/pest situation. Thus, lowering the conductivity of ioxynil formulations used against small broad-leaved weeds hidden in a winter cereal crop improved the ratio of electrostatic to hydraulic deposits on weeds from 0·59 to 0·82. Although electrostatic deposits were still smaller than hydraulic deposits, they differed in drop size, ion concentration of adjuvants and active ingredient, and gave better control of some weed species.

Effect of air assistance. Air-assistance was supplied to the electrostatic spraying system by a 5 hp engine driving a centrifugal fan linked to a manifold system, and thence along a series of ducts to produce a curtain of air at velocities of up to 30 $m\,s^{-1}$ behind the sprayers. The air is used to part dense crop canopies allowing a greater proportion of the charged spray to be deposited deeper in the canopy. In a spring barley crop at Zadoks growth stage 37–39 the addition of air at 30 $m\,s^{-1}$ to the Jumbo electrostatic sprayer significantly decreased deposits on the uppermost leaf and doubled those on the third leaf thus giving deposits in the lower crop canopy similar to those achieved with the hydraulic sprayer. In a separate trial in spring barley 'Tilt Turbo' applied at Zadoks growth stage 41 with an air assisted APE 80 sprayer gave similar control of mildew as an application with a hydraulic sprayer and a subsequent yield increase of 8% over the unassisted spray treatment.

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Effect of sprayer design. A charged vertical disc rotary atomizer has been developed which uses either direct charging at 10–30 kV, or inductive charging at 1–5 kV and produces a 140° flat fan spray with an 80 cm swath. Compared to the horizontal electrostatically charged rotary atomizers the increased vertical velocity imparted to the drops by the vertical disc projected the drops deeper into crop canopies, giving a two-fold increase in deposit on the third leaf of barley plants sprayed at Zadoks growth stage 37–39 and significant spray deposits on the fourth leaf where none could be detected from horizontal atomizers. The inductively charged system has the added advantage of using lower voltages and earthed spray tank which increases operator safety, an important consideration for commercial development.

Treatment of potato tubers. Work has also been started on new methods for applying pesticides to potatoes being loaded into store since existing methods are not completely satisfactory. The amount of spray intercepted by potatoes filling a roller table depends on tuber size; with seed-sized tubers, more than 30% of conventionally applied pesticide is deposited directly on to the rollers and is wasted. A Mantis CDA potato sprayer has been adapted to take an electrostatic charge and to change the spray distribution. Operating at 30 kV and with a disc speed of 1200 rpm the sprayer can atomize oil, water and wettable spray formulations at throughputs of 40–90 ml min^{-1}. In a spray booth fitted with electrostatic deflectors the spray pattern is further modified giving a more uniform distribution of chemical across the roller table, which can result in doubled deposit on test tubers as well as decreased operator hazard. Preliminary observations indicate that the increased spray deposits thus achieved can improve the effectiveness of fungicides used as seed treatments.

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

Pesticide distribution in plant and soil

Physicochemical properties determine the redistribution of chemicals after application to soils or plants and therefore have a major influence on efficacy. Current investigations include movement of chemicals in the phloem and xylem of plants and the effect of physical and chemical properties on the toxicity of soil insecticides and on the persistence and leaching of chemicals in soil.

Phloem translocation. Petiole injection of *Ricinus communis* solution-grown to the four-leaf stage and cut to retain only the upper pair of primary leaves as the sole source of phloem, complements techniques described previously.

Moderately lipophilic un-ionized compounds were found 24 h after treatment only in the leaf beyond the injection point whereas polar un-ionized compounds (oxamyl and aldicarb sulphone) also appeared in low concentration in the petiole, stem and apex and in the opposite leaf, whether its petiole was steam girdled or not, indicating export via the phloem and redistribution between xylem and phloem.

In a series of phenoxyacetic acids of similar pKa (about 3) there appeared to be an optimum lipophilicity for uptake from the petiole and export in the phloem. However concentration gradients in the phloem between the top and base of the stem were shallower for the more polar compounds than for those with optimum polarity for uptake.

A weaker acid, maleic hydrazide (pKa 5·6), with polarity similar to the most polar phenoxyacetic acid, had similar uptake from the petiole but was then distributed throughout the plant with no phloem concentration gradient in the stem and significant concentrations in the roots.

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Results with *Ricinus* are probably generally applicable since similar distributions of chemical were found in potatoes (using stem injection) and barley (using foliar application) although the results are less detailed because phloem cannot be sampled directly in these species.

Leaching in the field. Use of crop protection chemicals in autumn sown crops is now very important. The simulation model CALF has therefore been developed further to give better prediction of the movement of chemicals in soil in autumn and winter. Predictions from the model were compared with soil distributions measured up to the end of March of a range of representative chemicals applied at several dates during autumn and winter. The unmodified model can already predict movements under summer conditions accurately but in winter, when there is an overall large downward movement of water, time-dependent phenomena become important and must be simulated. By incorporating a variable adsorption coefficient which reflects increasing retention with time, predictability is regained, even under winter conditions. Thus, as predicted by the model, the moderately lipophilic weak acid chlorsulfuron, present in soil largely as the anion, was rapidly leached from the top 25 cm of soil while a comparably lipophilic neutral chemical and the more lipophilic chlortoluron remained largely in the top 10 cm.

Availability of soil insecticides. The toxicities of a range of organophosphates (*Rothamsted Report for 1982,* Part 1, 130) to the corn rootworm *Diabrotica balteata* were measured in soils at different moisture content. Toxicity decreased as soil became drier, an effect less pronounced in organic soils for all the chemicals tested and in mineral soils for the chemicals with highest air/soil ratios confirming the predictions from our earlier work.

Degradation of oximecarbamates in anaerobic subsoils. Like oxamyl (*Rothamsted Report for 1982,* Part 1, 137) aldicarb is broken down extremely rapidly in anaerobic soils, due to reduction by ferrous ions, but less rapidly in solutions of 250 $\mu g\,ml^{-1}$ ferrous ions at 30°C. The products from aldicarb in anaerobic soils are 2-methyl-2-methylthiopropionitrile and 2-methyl-2-methylthiopropionaldehyde, in approximately equal amounts, though only the former is obtained from incubations in solutions of ferrous ion. The first formed imino radical is presumably scavenged by Fe^{3+} in aqueous solutions leading to formation solely of the nitrile, but this process is much slower in soils permitting disproportionation of the imino radical to the nitrile and aldehyde products.

The breakdown of aldicarb sulphone is also much faster in anaerobic than aerobic soils, though still slow ($t_{1/2}$ up to 35 d at 20°C). This finding could be important as aldicarb sulphone is the most persistent residue from aldicarb and has been found in ground water in the USA (With J. H. Smelt, Institute for Pesticide Research, Wageningen).

(Briggs; Bromilow, Chamberlain, Chen Qui Fang, Evans, Farnham, Nicholls, Richards, Rigitano, Sengupta, M. Williams).

Staff of the Department and the Chemical Liaison Unit

In September this year, Dr Michael Elliott, CBE, FRS, retired after a uniquely distinguished career at Rothamsted which started in 1948. His substantial contribution to pesticide science has been widely recognized by industry and the adacemic community, culminating in the honour paid to him at the recent International Conference on insect control chemistry. He remains in the Department for a further year as an Honorary Scientist.

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F. T. Phillips and Kate E. O'Dell retired after 24 and 12 years' service respectively. Jean C. White took over Mrs O'Dell's duties. Lindsay E. Cooper resigned and was replaced by Sarah Perryman, and Valerie J. Church transferred to the Plant Pathology Department. Pauline Dand and A. Peel were appointed to temporary posts financed by the AFRC New Initiatives Scheme and by BTG.

Among visitors welcomed to the Department were H. Coskun, Regional Plant Protection Research Institute, Ankara; Professor A. Hassanali, International Centre of Insect Physiology and Ecology, Nairobi; C. E. Mantilla, Centra Internacional de Agricultura Tropical, Colombia; Julia Morgan, BASF, Hadleigh; Dr D. Sengupta, Pesticide Development Programme, India and Mr Zhang Zhong-ning, Institute of Zoology, Academia Sinica, Beijing, China.

G. G. Briggs visited the USA at the invitation of the American Chemical Society and R. H. Bromilow lectured on translocation of pesticides in plants at several centres in the USA.

M. Elliott gave an invited paper at Jornadas de Productos Fitosanitarios, Barcelona. D. W. Hollomon visited laboratories in Holland, West Germany and Belgium and attended the 36th International Symposium on Crop Protection at Ghent; he also attended the EPPO Conference on Fungicide Resistance in Brussels. J. A. Pickett gave lectures at the Universities of Lund and Gothenburg in Sweden. He was invited to present a paper at the Royal Society of Chemistry Conference, 'Chemistry for Development in the SADCC Region' in Zimbabwe; he also visited the ICIPE in Nairobi to discuss possible collaborative work, and other laboratories. A. D. Rice was invited to visit the headquarers of Hoechst AG in Frankfurt, R. M. Sawicki and A. W. Farnham organized the symposium on Resistance to Pesticides in Arthropods at the XVII International Congress of Entomology, Hamburg; Margaret M. Blight, D. C. Griffiths and J. A. Pickett also contributed to the Congress. R. M. Sawicki also attended conferences in Alexandria, Algiers and Abidjan. On private visits to New Zealand and South Africa, Margaret M. Blight and J. H. Stevenson gave lectures and visited research centres.

Members of the Department made substantial contributions to conferences and meetings in this country, notably the symposium on Pyrethroid Insecticides in the Environment, at Southampton (Session Chairman: N. F. Janes; Organizing Committee: J. H. Stevenson); the 1984 British Crop Protection Conference (Vice-Chairman of Programme Committee: A. L. Devonshire; Poster Manager: A. W. Farnham) at which 13 posters were presented; and the Conference on Recent Advances in the Chemistry of Insect Control at Cambridge University (Session Chairman: M. Elliott; Organizing Committee: J. A. Pickett; Proceedings Editor: N. F. Janes) where M. Elliott gave a keynote paper and was presented with the first Royal Society of Chemistry's Fine Chemicals and Medicinals Award to mark his retirement from Rothamsted staff.

NEMATOLOGY DEPARTMENT

Generally, the major nematode pests in agriculture are those forms showing greatest adaptations to the parasitic habit. Of these, various species of cyst nematode and a multiplicity of host races of the stem nematode are the most notable in the United Kingdom as agricultural pests. It is towards the control of these species, and the alleviation of the crop losses that they cause, that most of the Department's work is directed. Reflecting concern for the environment, much of this effort is directed towards improving the efficacy of pesticide use and to developing alternatives to chemical control. Fortunately the specialized biology of such highly adapted parasites presents unique opportunities for the development of control measures and it is to one of these,

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resistance and tolerance to potato-cyst nematodes in potatoes, that the major part of this year's report is devoted. Some other aspects of the Department's work are described briefly.

The main elements of the Department's long term programme include: experimental taxonomy of species complexes and pathotype systems of agricultural importance, including biochemical taxonomy; the continuing provision of a taxonomic reference laboratory; exploration of computer applications in handling taxonomic information; studies of host parasite relations, especially of cyst nematodes; exploration of nematode physiology and behavioural triggers with the aim of developing novel control measures; integrated pest management of cyst and stem nematodes with nematicides, resistant and tolerant cultivars; manipulation of natural fungal pathogen populations to enhance nematode control and the development of biological control agents. Work on aspects of tropical nematology will continue under short term contracts.

Potato cyst nematode: resistance and tolerance

Resistance. For proper understanding of these studies some definitions are necessary. Pathotype and host race are defined in *Rothamsted Report for 1983,* 109. Virulence, used later in this report, is defined as the ability to break resistance and produce cysts on a resistant host. Thus the term is used in a sense different from that in plant pathology but its adoption avoids introduction of new jargon.

Four sources of resistance are of importance in United Kingdom potato breeding programmes. Of these, two are known to be dominant major genes: H_1, from *Solanum tuberosum* ssp. *andigena* CPC1673 conferring resistance to *Globodera rostochiensis* pathotype Ro1 and H_2, from *S. multidissectum* conferring resistance to *G. pallida* pathotype Pa1. The remaining two sources are believed to be oligogenic: *S. vernei* CPC2487 and 2488, and *S. tuberosum* ssp. *andigena* CPC2802, both conferring quantitative resistance to *G. pallida.*

Jones and Parrott (*Nematologica* (1981) **27,** 372–384) demonstrated evidence for a gene-for-gene relationship between gene H_1 and a hypothetical virulence gene in *G. rostochiensis* and between gene H_2 and a hypothetical virulence gene in *G. pallida.* Recent work in the Department (*Rothamsted Report* Part 1 for 1978, 179–80; 1979, 144–145; 1980, 156–157; 1981, 163; 1982, 163–164; 1983, 116) has been concerned with resistance to *G. pallida* derived from *S. vernei.*

Selection for virulence. Resistance in *S. vernei* and its hybrids has been attributed to unspecified 'polygenes' with the assumption that it is 'horizontal' or race-non-specific, and with the implication that the resistance is durable, i.e. will not break down in field conditions. Turner and Stone tested this hypothesis by examining the durability of ex *vernei* resistance to *G. pallida,* the attribute tested most easily by experimentation. Selection of potato cyst nematode populations on the resistant ex *vernei* hybrids which are used to differentiate *G. pallida* pathotypes (see *Nematologica* (1977) **23,** 333–339) resulted in an increase in virulence of these populations on the resistant clones on which they were selected; the increases are in some cases very considerable after selection through only six generations. These tests were done in the glasshouse with cysts developing in each generation harvested and used as inoculum for the next. Thus no dilution of the selection process by unhatched eggs carried over from one generation to the next occurred, nor was the fitness of the highly selected individuals produced in the later generations tested against that of less highly selected ones.

Effects on nematode development. Numbers of juveniles invading root systems have been found not to be clearly related to resistance but in both gene H_1 mediated

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resistance to *G. rostochiensis* Ro1 and ex *vernei* resistance to *G. pallida* progress of nematode development is slowed. This appears to be a major effect of resistance and its contribution to limiting nematode multiplication appears greater than that of the decreased hatching activity (compared with that induced by susceptible potato plants) in ex *vernei* clones or to the emergence of juveniles from resistant roots after invasion. In ex *vernei* clones the degree to which nematode development was slowed varied with clone and nematode population, and is consonant with the resistance being mediated by several genes, with different numbers of genes being involved in the responses to different nematode phenotyes. (Stone and Dr S. J. Turner, Department of Agriculture, Northern Ireland)

Histology of resistant responses. Changes in roots of resistant potato plants with either gene H_1 or ex *vernei* genes conferring resistance to *G. rostochiensis* pathotype Ro1 and *G. pallida* respectively were examined from 24 h to 10 days after invasion in potato roots grown *in vitro*. Once the invading second-stage juveniles have penetrated the roots and moved to lie with the head adjacent to the stele, initiation of the feeding site begins by breakdown of cell walls of inner cortical and stelar cells adjacent to the nematode stylet to form a syncytial complex. In the case of major gene resistance to *G. rostochiensis* cells surrounding the developing syncytium undergo a hypersensitive response which presumably limits movement of nutrients from plant to nematode. This is accompanied by degenerative changes in the ultra-structure of the syncytium. In the case of ex *vernei* resistance to *G. pallida* the hypersensitive response to the developing syncytium varies with potato clone and nematode population, ranging from a response similar to that to *G. rostochiensis* in roots of plants with gene H_1, to a response in which the hypersensitive reaction is absent but degenerative changes nevertheless occur in the developing syncytium. The differences between the two types of resistance and the variable condition of the response in ex *vernei* plants accords with the assumptions about the genetic bases of the two types of resistance. Root cells surrounding the invading nematodes undergo a hypersensitive response (distinct from the wound response to the juveniles passage through root tissue) but this does not appear to inhibit initiation of feeding sites. This early response is common to both types of resistance and the main components of both types appear to involve reactions to the initiated feeding site rather than to the nematodes themselves. The elicitor or elicitors of this major component of resistance may be a change or changes in the plant cells modified to form feeding sites rather than components of the nematode cuticle or nematode excretions or secretions. (Stone, Rice and Dr B. S. C. Leadbeater, University of Birmingham)

***The nature of resistance to* G. pallida.** The hypothesis that cyst nematodes and their hosts have coevolved argues that resistance and virulence are most likely to be mediated by genes operating on a gene-for-gene basis. Such genes have specific action, the effect of a particular resistance gene in the host being negated by the effect of a particular virulence gene in the parasite. Such specific action implies that single or few genes are involved, i.e. major or oligogenes and the great majority of examples of resistance for which the genetic basis has been investigated prove to be conferred by genes of this type. Ex *vernei* based resistance to *G. pallida* is unusual in that the resistance has been held to be truly polygenic and race-non-specific. However, *S. vernei* hybrids are used to differentiate pathotypes of *G. pallida* although the differentiation is not precise (see below). Because *S. vernei* resistance differentiates populations of *G. pallida* in this way, it cannot be race-non-specific. The fact that loss of durability of this resistance occurs through rapid selection for virulence in *G. pallida* populations, further refutes the hypothesis and suggests that ex *vernei* resistance to *G. pallida* is indeed based upon oligogenes participating in gene-for-gene relationships. This interpretation is in accord-

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ance with the view that both so-called horizontal and vertical resistance in plants involve gene-for-gene relationships as proposed by J. E. Parlevliet and J. C. Zadoks (*Euphytica* (1977) **26,** 5–21). (Stone and Dr S. J. Turner, Department of Agriculture, Northern Ireland)

Implications of the gene-for-gene hypothesis. The major implication of the gene-for-gene hypothesis applied to ex *vernei* resistance to *G. pallida* is that such resistance will not be durable. This has been demonstrated experimentally and modelling of the 10% per annum increase in virulence observed in Turner's and Stone's experiments suggests that breakdown of resistance will eventually occur in the field if potato cultivars with ex *vernei* resistance to *G. pallida* are grown repeatedly. Applying the population model of Jones and Perry (*Journal of Applied Ecology* (1978) **15,** 349–371) and assuming an initial nematode population of 50 eggs g^{-1} soil, a hypothetical resistant clone grown in a four course rotation with non-host crops in intervening years and a 10% increase in virulence each time the resistant clone is grown, predicts that post-harvest populations of the nematode at first decline but then increase to nearly 200 eggs g^{-1} soil within eight rotations. A similar situation may develop with the resistance to *G. pallida* found in *S. tuberosum* ssp. *andigena* which, although originally believed to be conferred by a major gene, is now believed to be oligogenic (Dr A. J. Thomson, Plant Breeding Institute, personal communication). Although modelling of such changes provides useful predictions these must be tested against experimental observations, especially in field conditions where factors such as delayed hatch and changes in fitness may exert an influence. Such experimentation must, however, be prolonged. Microplot experiments beginning at Rothamsted in 1985 are not expected to yield results for between five and eight years. Meanwhile, the dangers of uncontrolled introduction of cultivars partially resistant to *G. pallida,* which may facilitate rapid selection of virulent field populations, must be considered.

Pathotypes. The current European scheme for pathotyping potato cyst nematodes was proposed by Stone and European colleagues (*Nematologica* (1977) **23,** 333–339) to provide a common nomenclature for European nematologists, plant breeders and advisory workers. It embodied the disadvantages as well as the advantages of the separate Dutch, German and British schemes. Proper definition of pathotypes requires that each one is defined against identified resistance genes. The current potato cyst nematode scheme is scientifically unsound because pathotypes Pa2 and Pa3 of *G. pallida* and Ro2, Ro3 and Ro5 of *G. rostochiensis* are defined by performance on differential clones of unknown genetic constitution. The plastic nature of virulence in *G. pallida* populations demonstrated by selection experiments demonstrates the unsatisfactory nature of the distinction between pathotypes Pa2 and Pa3. Such pathotypes are in fact merely artefacts of the test procedure in which type populations of *G. pallida* were held to behave differentially in a distinctive way on ex *vernei* potato hybrids of different but unknown genotypes. A survey of British *G. pallida* field populations supports this contention. Of 100 *G. pallida* field populations tested, 33 were impossible to attribute to either Pa2 or Pa3 alone while in many of the cases where a single attribution was made the response was equivocal (Stone, Parrott and members of the ADAS Potato Cyst Nematodes Working Party). Consequently, it is proposed that pathotypes Pa2 and Pa3, being artefacts of no useful application, be abandoned. A similar recommendation is made for *G. rostochiensis* pathotypes Ro2, Ro3 and Ro5, although as yet undetected in the United Kingdom. (Stone and Parrott)

Tolerance. Some potato cultivars and plant breeders' selections resistant to potato cyst nematodes have proved intolerant of nematode attack. Intolerance is defined as suffering

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an undue yield loss as a result of nematode invasion and can occur in resistant potatoes because resistance inhibits nematode establishment, maturation and reproduction on the potato plant, not invasion. Apparently some types of potato are more severely damaged than others by the invasion process. Screening for tolerance by assessment of potato tuber yields is not possible in tests with plants grown in small pots in the glasshouse. Consequently, intolerance has been detected only at a late stage when promising plant breeders' lines have been subjected to extensive field trials. Furthermore, tolerance and intolerance are not expressed consistently, being affected by environmental factors.

Existing cultivars differ markedly in tolerance and even susceptible cultivars differ in their tolerance to nematode attack. Recent work at Rothamsted has been designed to establish what factors contribute to tolerance and to determine how they might be used in tests before the field stage. (*Rothamsted Report,* Part 1, for 1980, 155; 1981, 159–160; 1982, 159–160; 1983, 115–116).

Efficiency of water use by potato plants initially increases with nematode attack but decreases later in the growing season, much more so in intolerant than in tolerant clones. Because calcium enters plant roots by mass flow rather than active transport the amount taken up is an indicator of the quantity of water passing through the plant. Calcium is retained in plant tissues and the percentage of calcium in plant dry matter is related to water use efficiency. However, there is not a simple relationship between calcium content of dry matter and the efficiency of water use by a potato plant parasitized by potato cyst nematode. This is because calcium enters roots chiefly where secondary thickening of the endodermis is incomplete and the amount of secondary thickening varies with potato type as well as age and growth state of the root system. Additionally, potato cyst nematodes disrupt the endodermis and may permit increased entry of calcium by mass flow. The relationship between calcium content and water uptake varies between cultivars and with the presence or absence and level of nematode infestation. Furthermore, the maturation behaviour of a potato plant also affects this relationship. Early maturing potatoes divert assimilate to tuber formation at a relatively early growth stage with a consequent reduction in new leaf formation. The transpiration stream continues to bring calcium into the leaves and thus the overall foliar calcium content rises relative to that in later maturing potato plants which form new leaves later in the season, initially with low calcium content. However, when cultivars of the same maturity date are tested together it is possible to show some correlation between calcium content and tolerance. Calcium is always increased by nematode attack and potassium and phosphorus generally decreased. The ratio of calcium to the other elements is greater in less tolerant plants and this also allows some estimation of tolerance, but only crudely.

Other studies in relation to tolerance have included growth analyses in the field and in root observation boxes, the relationship of soil temperature to damage, interactions with disease organisms and the build up of stress metabolites after nematode infestation. A number of factors associated with tolerance have been identified.

Maturation dates. Yield of early maturing potato types is determined at the time of maximum nematode invasion but later-maturing forms do not initiate tubers until the main phase of nematode invasion is over. Main crop potatoes are therefore still in the vegetative phase at the time of maximum invasion, with vigorous root growth which can offset nematode damage. In general, then, early potatoes are less tolerant of nematode attack, a fact which can be shown readily in fresh trials.

Root proliferation. Growth analyses of field-grown plants show that cultivars of widely different tolerance all have similar ratios of shoot weight to root weight on any given date when grown together in the same experiment and that this ratio is decreased by a similar

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amount by nematode infestation in all. Root weights of very intolerant cultivars are decreased by nematodes, those of tolerant cultivars are unaffected while those of some very tolerant cultivars are actually increased. This plant root response to nematode attack is reflected in top growth (because shoot weight/root weight ratios behave similarly) and in yields.

Root system form. Large observation boxes were used in making weekly tracings of root system development of tolerant and intolerant potatoes. The very tolerant cultivar Cara has a much more extensive root system than less tolerant cultivars and the Cara root system extends deeper into the soil profile, below the zone of maximum nematode attack (0–30 cm). Water stress increases yield loss associated with potato cyst nematode and such a deep root system will exploit more abundant water reserves. Measurements by neutron probe moisture meter of water use at different soil depths by different cultivars show that more tolerant cultivars are able to exploit deep water reserves more effectively than less tolerant ones.

***Interactions with* Verticillium dahliae.** There is an interaction between infestation of potato roots by potato cyst nematode and infection with *V. dahliae* (*Rothamsted Report for 1967,* Part 1, 152). Damage is more severe when both organisms are present than the sum of damage caused by similar levels of the two organisms present separately. Distribution by *V. dahliae* in the UK is sporadic and its presence in a field site may contribute to poor tolerance. Furthermore, the nature of the interaction between the two organisms varies with cultivar; Maris Anchor can be infected by *V. dahliae* in the absence of potato cyst nematode but Maris Peer is infected only if nematodes are present in the roots while Pentland Javelin is not infected even when both organisms are present.

Other effects. Many of the differences in tolerance between cultivars can be explained by the mechanisms discussed but there are almost certainly other effects such as alteration of source/sink relationships in the plant, and nematode induced changes in levels of growth regulating hormones. Abscisic acid level was found to be approximately doubled in plants grown in field plots with large nematode populations, with the greatest increase occurring in the least tolerant cultivars. Proline levels are known to increase in water stressed plants and were also shown to increase in nematode infested plants, but the changes in proline content associated with plant age were much greater with those associated with nematode attack.

Tolerance tests. Simple tests for tolerance, based on measurement of a single attribute, cannot take account of all the factors involved. The most useful test, therefore, is a field trial repeated at a number of sites but this is time-consuming, expensive and requires large quantities of potato seed tubers. Thus it cannot be applied in the early stages of potato selection. The single most important feature contributing to tolerance is probably root vigour and ability of roots to regrow after nematode attack. At present the most practical recommendation for the development of tolerant cultivars is to select for root system vigour at all stages of a breeding programme. (Evans)

General aspects

Control measures. In this year's report attention is given to continuing investigations with stem nematodes *(Ditylenchus dipsaci),* a report of the large amount of work on integrated control of cyst nematodes being deferred. Because it is a foliar parasite *D. dipsaci* presents problems somewhat different from those encountered in the control of cyst nematodes (*Rothamsted Report for 1983,* 113).

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Control of stem nematode

Resistant cultivars. The susceptibilities of ten cultivars of red clover to seven populations of the red clover race and four cultivars of lucerne to 11 populations of the lucerne race were assessed in glasshouse tests. Plants were scored for visible symptoms and multiplication of the nematodes in the plants was determined. Susceptible cultivars of red clover (Redhead, Mt Calme and Leisi) allowed all populations to multiply freely. Amongst seven supposedly resistant cultivars Sabtoron was most resistant, followed in descending order by Norseman, Quin, Rittinova, Grasslands Pawera, Renova and Britta. Seedlings of a cultivar differed greatly in their reactions to inoculation and, except for Sabtoron, all resistant cultivars allowed multiplication of at least one of the nematode populations used. Similarly, resistance in the lucerne cultivars Euver, Lifeuil and Vertus to lucerne race was unsatisfactory. Lifeuil and Euver were as susceptible as the susceptible cultivar Europe, and Vertus was only resistant to four of the 11 populations used. Resistance to stem nematode in the supposedly resistant cultivars of red clover and lucerne currently available to British farmers is far from satisfactory. In white clover no resistance to two populations of white clover race was detected in the cultivars Rivendel, Kersey, Gandalf and Milkanova. Tests of susceptibility of other dicotyledonous crops is in hand but attempts to assess efficacy of resistance in oats is limited by lack of a reliable method of inoculating monocotyledons in the glasshouse. (Whitehead, Fraser and Nichols)

Nematicides. At Rothamsted, in soil inoculated with lucerne race of stem nematode, damage was prevented and herbage yields of the susceptible lucerne cultivar Europe were very greatly increased after application of carbofuran applied to the seed furrows in spring 1983, followed by electrostatic or hydraulic spray applications of thiabendazole, carbendazim or dimethoate applied to the young foliage after the second cuts in 1983 and 1984. Foliar sprays may therefore control stem nematode attack in lucerne but further work is needed to confirm this, to determine optimal dosages and to ensure that the herbage does not contain residues. In winter beans (cv. Throws MS) carbofuran applied to the seed furrows very significantly reduced the proportion of stems infested when the seed was drilled into the ground but not when it was ploughed in. Aldicarb failed to control stem nematode with either method of sowing. (Whitehead, Tite, Fraser, Nicholls and Penn)

At Grasslands Research Institute carbofuran, and to a lesser extent aldicarb, applied to the seed furrows at sowing dramatically improved establishment of four irrigated cultivars of white clover in land infested with white clover race of stem nematode. (Whitehead with R. O. Clements and G. Lewis, GRI)

Seed borne infestations. Stem nematode infestations of pre-basic, basic and certified stocks of field bean seeds remain a potential source of infesting clean land as well as reducing yields. Of 96 samples of certified seed eight were found contaminated. Ninety-eight samples of various varieties of grain and fodder pea were also examined but none found infested. (Hooper and Cowland)

Biological control of cyst nematodes. Work in the Department on biological control has continued to focus on the parasite *Verticillium chlamydosporium*. The pathogenicity of six different isolates was compared quantitatively *in vitro* by exposing eggs of cereal cyst nematode *(Heterodera avenae)* on 2% water agar to different densities of conidia and estimating the number of eggs killed after three weeks at 12°C. The most pathogenic isolate had a $\log_{10}$ LC50 of 3·2 conidia cm^{-2} while for the others it was >6. At all densities of conidia fungal hyphae grew in contact with nematode eggs but there was a

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positive correlation between conidial density and number of eggs killed; apparently a substantial inoculum is essential to ensure infection.

V. chlamydosporium must grow in soil if it is to make contact with sedentary nematode females and eggs and the fungus must successfully compete with the natural microflora. In general fungi added to soil require an energy source to overcome fungistasis and become established. However, survival in soil greatly depends on the type of carrier used to introduce a fungus: precolonized intact grain, successfully used to introduce other fungi, supported growth in soil by *V. chlamydosporium* only in sterile conditions. In non-sterile soil the grain may have favoured the microbial competitors of *V. chlamydosporium*. Nevertheless, when introduced on attapulgite clay granules into soil the fungus could be re-isolated after six months while the growth of colonies on corn meal agar was little affected by a wide range of pH or by water potentials in excess of permanent wilting point. Thus there are indications that introduced *Verticillium* can tolerate a wide range of soil conditions.

In soils showing natural decline of cereal cyst nematode it is estimated there are approximately 1000 *V. chlamydosporium* propagules g^{-1} soil. It has proved impossible to introduce an equivalent amount of inoculum with quantities of the carriers grain, vermiculite, perlite or attapulgite clay in quantities that could be practically applied on a field scale. However, large quantities of inoculum can be produced in liquid culture but the best method for incorporating the fungus into the soil has yet to be determined. (Kerry, Crump, Irving and Hornsey)

Effects of nematodes on oilseed rape. Following further reports of damage to oilseed rape crops caused by the brassica cyst nematode *(Heterodera cruciferae)* 47 oilseed rape crops were examined. Beet cyst nematode *(H. schachtii)* also attacks oilseed rape, so fields of winter rape were selected in three areas: south Lincolnshire, where vegetable brassicas but little sugar beet are grown; north Norfolk, where sugar beet but few vegetable brassicas are grown; Cambridgeshire and Huntingdonshire where both vegetable brassicas and sugar beet are grown. Soil and root samples were taken from each field in April for estimations of cyst nematodes and root lesion nematodes (*Pratylenchus* spp.). Incidence of the cyst nematodes was low but root-lesion nematodes were found in roots from 44 out of 46 sites and ten contained more than 200 *Pratylenchus* g^{-1} root. Infestation of rape crops by root lesion nematodes may be widespread and it is of interest that reports of damage by *Trichodorus* spp. have been received. The effect on extensive cultivation of oilseed rape posed by cyst nematodes may extend to effects of these vermiform species. (Evans and Spaull)

In continuing investigations of the effect of stubby-root nematodes (*Trichodorus* and *Paratrichodorus*) on crops grown on light, sandy soils a site at Holme-upon-Spalding moor, North Humberside infested with an estimated 8290 trichodorids (mostly *T. cylindricus*) litre^{-1} soil and 3920 litre^{-1} soil of other plant parasitic nematodes was treated in autumn with oxamyl at varying rates. The regression of winter oilseed rape yield upon nematode numbers in early spring showed that the trichodorids accounted for the greatest variance and indicated that a yield loss of 0·13 t ha^{-1} rape seed (92% dry matter) may be expected for every 1000 trichodorids present in a litre of soil, representing a financial loss of approximately £35 ha^{-1}. (Spaull and Mewton)

***Infectivity of* Globodera *juveniles*.** The potential infective life of second-stage juveniles of *G. rostochiensis* and *G. pallida* is influenced by the extent of their lipid reserves at the time of hatching and the subsequent rate of utilization of lipids before feeding commences. Lipid utilization by both species was estimated by measuring the amount of oil red O stain taken up in juveniles, using scanning microdensitometry. Utilization after

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stimulation by host root diffusate but before hatching was found to reduce the infectivity and retard development rates of late hatching juveniles. The effect was more marked in *G. rostochiensis* than *G. pallida* and this probably reflects differences in their hatching patterns. Presence of potato root diffusate increased the rate of the lipid utilization by hatched juveniles five-fold compared to juveniles kept in artificial tap water. The depletion of lipid reserves is correlated both with temperature and moisture content of soil. Juveniles with depleted reserves had a reduced rate of movement in mobility tests and also showed a disorientation. Because field populations of potato cyst nematode may vary in their physiological state through age and other factors, measurement of lipid reserves may provide a more accurate assessment of potential for damage than simple viable egg counts. (Robinson and Perry with Dr H. J. Atkinson, University of Leeds)

***Cold tolerance in* Globodera rostochiensis.** *G. rostochiensis* is believed to have evolved in the high Andes where soil temperatures are below freezing for substantial periods of the year. Furthermore, the nematode is now distributed in many parts of the world where low winter soil temperatures are encountered. Together with other cool climate nematodes, this species must have evolved cold protection mechanisms. A thermoelectric microscope stage which allows specimens to be observed as they are cooled at a constant rate of $1° \text{ min}^{-1}$ was used in observations of the super-cooling points of hatched and unhatched second-stage juveniles. Hatched juveniles can survive sub-zero temperatures by super-cooling when not in contact with water; the temperature at which 50% of the nematodes were killed (T_{50}) was −31·8°C. When frozen in water, free juveniles cannot survive ice seeding across the cuticle and concomitant freezing of their body contents. Unhatched juveniles in contact with water can survive freezing; after exposure to −20°C the percentage hatch from eggs was 65%. The eggshell apparently protects the juvenile from the external medium and juveniles survive by super-cooling in a manner similar to that of certain cold-hardy arthropods. Treatment of eggs with potato root diffusate for a period long enough to allow trehalose leakage from the egg fluid but not long enough to result in hatching did not adversely affect freezing survival, indicating that trehalose in the egg fluid does not act as an anti-freezing agent. (Perry with Dr D. A. Wharton, University of Wales, Aberystwyth)

***Functional morphology of* Pratylenchus *sense organs*.** The ultrastructure of the anterior sensilla of adult *Pratylenchus crenatus, P. penetrans* and *P. thornei* has been examined by scanning and transmission electron microscopy. The inner labial sensilla, which comprise two dendritic processes within a short, cuticle-lined canal, are believed to be combined chemo- and mechanosensory units, by structural analogy with arthropod sensilla. Apical pores of these sensillum canals are arranged around the prestoma and these sensilla may play an important role in the initiation of feeding responses at the surface of, and within, roots. The cephalic and outer labial sensilla are thought to be mechanoreceptive and each possesses a single dendritic process which ends embedded in the oral cuticle. The processes of the submedian outer labial sensilla differ from those of the lateral receptors as they curl around the cephalic processes. This condition is also present in other tylenchid species as well as soil-dwelling stages of animal parasitic Trichostrongyloidea and may enhance transduction of tactile stimuli encountered during locomotion in soil. The amphids are structurally the most complex of the sense organs and would appear to be the primary chemosensory organs. Each contains seven dendritic processes within a cuticle-lined canal. The processes of two further dendrites terminate within a secretory sheath cell at the base of the amphidial canal and may monitor ionic conditions in the receptor cavity. In addition to the cephalic sense organs, lamellate terminals of between six and eight 'accessory neurons' also have been identified amongst labial tissues but

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their role is uncertain. Parallel studies of juvenile stages of selected Heteroderidae are in progress. (Trett and Perry with Professor J. Green, Queen Mary College, University of London)

Interactions between nematodes and other organisms. Aldicarb applied to seed beds can improve yields of peas in a range of soil types and conditions but the effect cannot be attributed to suppression of any single organism. Examination of plant-parasitic nematodes and fungi associated with roots of pea plants growing in field sites with silt soil (Norfolk), loam (Suffolk) and heavy clay loam (Rothamsted) showed differences although root lesion nematodes, a root-rot fungus *(Fusarium solani)* and an unidentified myxomycete were the most common endoparasites at all sites. Treatment with aldicarb and the fungicide tolclofos-methyl to the seed bed at each site in the spring of 1984 confirmed interactions previously detected in pot and *in vitro* experiments. Increased weights of haulm and peas, increased nodulation and decreased *Pratylenchus* invasion were associated with aldicarb treatments at all sites. *In vitro* experiments have shown that nematode invasion decreases nodulation (*Rothamsted Report for 1983,* 111–113). *F. solani* was isolated more frequently from plants in aldicarb treated plots than in controls, confirming earlier findings that this nematicide removes some natural control of *Fusarium* invasion. However, aldicarb may control root-rot because in the lighter soils the symptoms were least in treated plots. Fungicide application increased numbers of *Pratylenchus* in roots at all sites. *In vitro* experiments have shown that growth of a grey sterile mycelium or of *Thielaviopsis basicola* on or in the root epidermis inhibited *Pratylenchus* invasion. These fungi, commonly present on pea roots, may confer some protection against root lesion nematodes. Evidence from this and previous years' work indicates that *Pratylenchus* and *Fusarium* are common factors in the root disorder of peas causing poor growth in Eastern England. Both decrease nitrogen fixation but because nematicide or fungicide treatments may increase their levels in pea roots, alternative control measures should be sought. (Green)

A population of *Aphelenchoides ritzemabosi* from chrysanthemum leaves has been successfully cultured through several generations for six months on a sterile fungus, the first report of this nematode feeding on a fungus. (Hooper and Cowland)

Taxonomy and morphology

Aphelenchoides. Isoelectric focusing on polyacrylamide and agarose gels of soluble proteins from various populations of *Aphelenchoides* spp. gave characteristic patterns distinguishing morphologically similar populations. The pattern from a particular population was the same whether the nematodes had been cultured on *Botrytis cinerea* or *Rhizoctonia solani.* Different populations of the same *Aphelenchoides* spp. produced very similar patterns. (Burrows and Hooper)

Spicules of various *Aphelenchoides* spp. have been examined by scanning electron microscopy. Each spicule consists of a single laterally flattened, ventrally curved plate; the dorsal edge being much thickened and the ventral edge somewhat less so. However, the dorsal and ventral edges do not form separate limbs as is so often figured in light microscope studies. Scanning electron microscopy emphasizes differences in spicule shape and patterning between species. (Hooper and Spratt)

Hexatylus. Females and juveniles of *Hexatylus viviparus* from soil from Broadbalk Wilderness, Rothamsted, readily reproduced on *in vitro* cultures of *Botrytis cinerea.* They are a parthenogenetic form, cultures being readily obtained from individual juveniles with sperm not observed within females. This population has the characteristic

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morphology of the genus including a short stylet with biphid basal knobs. But in ageing fungal cultures occasional males and ensheathed pre-adult females occurred. This pre-adult female has a longer, stouter, stylet with asymmetrical basal knobs and a different oesophageal morphology. Although the vulva and reproductive tract are not fully developed in these specimens, pre-adult females contained sperm, presumably from the males that are with them. These individuals have a superficial resemblance to the ensheathed infective females of the entomophilic nematode *Tripius sciarae* which parasitize larvae of mycetophilid flies. Although attempts to infect such larvae with the ensheathed *Hexatylus* females have been unsuccessful, morphology possibly indicates a close relationship between the fungal feeding *H. viviparus* and the insect parasite. (Hooper)

Cyst nematodes. Work on the differentiation of *Globodera* species parasitizing Compositae from those parasitizing Solanaceae continues (*Rothamsted Report for 1983,* 118). Further useful characters distinguishing *G. achilleae* from *G. pallida* have been found or confirmed and the morphological differences between species parasitizing Solanaceae and those parasitizing Compositae are believed to be sufficient to warrant their placing in separate genera. Isoelectric focusing of soluble proteins in polyacrylamide and agarose gel slabs has been applied to the distinction of potato cyst nemaode species and to other members of the *Globodera* complex. Restriction analyses of total DNA extracted from potato cyst nematodes has begun with the modification of a technique to extract efficiently high molecular weight DNA from *Globodera* juveniles. ECO R1 restriction fragment patterns for two populations of *G. rostochiensis* and one of *G. pallida* have so far been produced with differences in number and relative mobilities of their bands in electrophoresis. (Burrows and Stone)

Root-knot nematodes. The compilation of a wide range of characters to better differentiate members of this agriculturally important genus is now complete (*Rothamsted Report for 1983,* 117). Preparation of a handbook on the genus is now in hand and this will include descriptions, keys and guides to identification of all species as well as sections on the culture, extraction and preparation of specimens. (Jepson and Hoole)

Staff and visiting workers

Mrs Sybil Clark transferred from Rothamsted to the Food Research Institute, Norwich after 30 years' service in the Nematology Department; she will be greatly missed. Mr G. M. Gurr spent an extended period in the Department on secondment from NIAB in connection with joint work on partial resistance to potato cyst nematodes. The Department was host to the Association of Applied Biologists' Workshop on Criconematid Nematodes attended by 30 delegates and nine ADAS staff trained in the Department for a short period. There were numerous short term visitors from home and overseas including Dr L. A. Guskova and Dr V. P. Balaknina from the Soviet Union, who spent three weeks in the Department.

K. Evans, S. B. Jepson, R. N. Perry and M. P. Robinson attended the First International Congress of Nematology, Guelph, Canada and Evans participated in the Sixth Cyst Nematode Workshop, Beltsville and also visited Cornell University. B. R. Kerry presented invited papers at the First International Workshop on Biological Control of Nematodes, Lima, Peru. A. G. Whitehead visited nematological centres in Switzerland and Greece, and gave an invited paper at a European Plant Protection Organization meeting in Crete. A. R. Stone gave an invited paper at the EPPO Workshop on Cyst Nematodes, Münster, West Germany and visited nematological laboratories in The Netherlands. C. D. Green visited the Christian-Albrecht University,

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Kiel, West Germany. During the year members of the Department contributed to several scientific meetings in the UK.

PLANT PATHOLOGY DEPARTMENT

The Department has vigorous programmes of work studying the pathogens and the diseases they cause in break crops, especially *Vicia* beans and oilseed rape, potatoes and cereals, plus associated studies on fundamental properties of viruses and viral infections, aerobiology, microbial contamination of stored grain and hay, and medical aspects of plant pathology. The Department makes a substantial contribution to the multidisciplinary studies of beans, wheat, barley and potatoes, and this work is reported under Multidisciplinary Agronomy. These studies have confirmed the central role of disease in crop production systems. This importance is reflected in the stimulus from members of the Department for multidisciplinary studies of oilseed rape and the large contribution from Plant Pathology to straw disposal work, both of which started this year. Our efforts are directed, through an understanding of the basic biology of the crop/disease interaction, to safe, timely and environmentally acceptable control of disease of arable crops. Some aspects of this work are reported below.

Diseases of break crops

Diseases of oilseed rape

Dry weather and light leaf spot. Despite prolonged dry weather in spring 1984 natural infection by light leaf spot *(Pyrenopeziza brassicae),* a principally splash-dispersed pathogen, continued to develop to give severe symptoms on upper leaves and bracts of cv. Jet Neuf at Rothamsted during stem extension and flowering, with much infection later on pods. Severity was scored on a 0–3 scale corresponding to 0 no infection, 0·1 trace infection first signs of acervuli, 1<10% area affected, 2 10–50% area, and 3>50% area affected; 48% of plants were infected, 6% with >10% total leaf area affected at the beginning of April and 98% by mid-May with 27% plants with >10% of total leaf area lesioned. By mid-May an average of eight leaves were infected on each plant (59% of all leaves assessed) with a mean severity score of 1·3. By mid-June 85% of stems were infected and 84% of plants had some infected pods. The mean severity scores for the topmost seven bracts and leaves (in descending order down the plant) on 20 June were 0·4, 0·8, 1·0, 0·9, 1·6, 1·7 and 1·9; the mean score for all pods was 0·5. By 10 July 95% of terminal branches carried some infected pods and 16% of these had at least a quarter of the total pod surface area infected; the mean pod area infected per terminal was 11%.

These results, in an unusually dry season, challenge the widely held view (*Ministry of Agriculture, Fisheries and Food (1982), Booklet 2387*) that light leaf spot is checked and ceases to be important in a dry spring. Obvious symptoms in this crop were first seen in March; fungicides (prochloraz and prochloraz+carbendazim) applied in April gave good control, particularly of symptom severity, but little increase in yield. Since other work (Rawlinson, Muthyalu & Cayley, 1984) has shown the benefits to yield of fungicides applied in autumn, these results indicate that damage by the pathogen may be done during autumn and winter when primordial tissue of leaves and floral structures may be infected but symptoms are not obvious. (Rawlinson; Muthyalu)

Mustard oils, fungicides and disease. One object in oilseed rape breeding is the production of winter hardy cultivars of *Brassica napus* with low concentration of glucosinolates in seed. This may result in cultivars with decreased amounts or types of mustard oils and other glucosinolate products, some of which are fungitoxic and also

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attractants for insects and slugs, in plant tissues. A large change in chemical composition could alter the spectrum and severity of pest and disease attack.

Gas liquid chromatography analyses in 1982–83 on plants grown from seed with glucosinolate content ranging from >100 to <10 μmol g^{-1} defatted meal showed large differences between cultivars in amounts of volatile N-containing compounds in leaves. In field plots downy mildew *(Peronospora parasitica)* developed early in all cultivars but infection was least on cultivars with >100 μmol glucosinolate g^{-1}, intermediate on cultivars with 15–40 μmol g^{-1} and most on a cultivar with <15 μmol g^{-1}. The glucosinolate products in leaves of all cultivars reached a maximum in mid-February, coincident with least downy mildew in all cultivars.

A field experiment on a high glucosinolate (cv. Norli) and a low glucosinolate cultivar (a breeding line) tested the effects on disease and yield of sprays of two experimental slow release formulations of isothiocyanates: allyl isothiocyanate (I) and phenylethyl isothiocyanate (II). Formulations I and II at 150 and 240 g ha^{-1} were compared with a conventional systemic fungicide, benomyl (0·5 kg a.i. ha^{-1}) and elemental sulphur (10 kg ha^{-1}). All treatments were applied on 30 November 1982 and 12 April 1983, using Rothamsted's APE 80 electrostatic rotary atomizer, except for sulphur which was applied by hydraulic sprayer. Disease on field plots was ensured by scattering finely chopped rape straw over each plot after sowing (26 August). Much light leaf spot and canker developed during winter and spring especially in the low glucosinolate cultivar; all plants of this cultivar were killed by the end of April. By 22 March all treatments of the high glucosinolate cultivar had decreased incidence of canker from 63% plants lesioned in untreated plots to 30% with benomyl, 47% and 37% with isothiocyanates I and II and 33% with sulphur. By 13 July all treatments had prevented or delayed spread of light leaf spot to pods; 23% of pod area was infected in untreated plots, 3% with benomyl, 15% and 10% with isothiocyanate formulations I and II and 9% with sulphur. Yields from untreated, benomyl, isothiocyanate formulations I and II and sulphur-treated plots were 2·05, 2·60, 2·29, 2·47 and 2·46 t ha^{-1} respectively (SED=0·246, 8 d.f.). Thus comparatively small amounts of isothiocyanate formulations had a similar effect on disease and yield to that achieved by the 40-fold greater quantity of sulphur, although neither formulation I nor II gave a yield increase as great as benomyl. The phenylethyl isothiocyanate formulation II was more active than the allyl in this experiment. Laboratory experiments confirmed that allyl-, phenyl-, phenethyl-, ethyl-, butyl- and cyclohexyl-substituted isothiocyanates at concentrations ≤10 ppm in agar or in the vapour phase all inhibited mycelial growth and spore germination of *Pyrenopeziza brassicae*.

In 1984 field experiments compared the effects of a single electrostatic application (on 4 or 13 April) of isothiocyanate II (250 g ha^{-1}) with the fungicides prochloraz (400 g a.i. ha^{-1}) and prochloraz+carbendazim (400 and 150 g a.i. ha^{-1}) on cv. Jet Neuf (sown 25 August). From March light leaf spot was the principal disease in both experiments and by 30 May all plants and a mean of 62% leaves were infected in untreated plots. All treatments decreased incidence and severity of symptoms on leaves in May, slightly decreased severity but not incidence of infection on pods in July and slightly increased yield. Most yield increase, 0·21 t ha^{-1} (4·6%), was on plots sprayed on 4 April with isothiocyanate formulation II.

These preliminary results indicate that there may be benefits in adding certain mustard oils to the growing crop, even on high glucosinolate cultivars such as Norli and Jet Neuf and that the consequences of breeding to influence pest and disease attack should be investigated before the widespread introduction of new cultivars. (Rawlinson; Muthyalu and Poole with Cayley and Hulme, Chemical Liaison Unit, and Pickett, Insecticides and Fungicides Department)

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Diseases of grain legumes

Chocolate spot of beans. The progress of a chocolate spot *(Botrytis fabae)* epidemic in winter-sown field beans was followed by monitoring numbers of *B. fabae* conidia using vertical cylinder and horizontal slide samplers. To examine the roles of wind and rain in dispersal of conidia during flowering and pod set, a Burkard volumetric sampler and pieces of fixed photographic film were also placed in the crop between mid-May and mid-July. Slides and cylinders collected most conidia after stem extension. The largest spore catch (2200 cm^{-2} day^{-1}) was by slides from 18–21 June during wet weather (17–21 June, 10·1 mm rain). Numbers of spores collected usually increased in wet weather and declined when it was dry. There was no obvious correlation between numbers of spores collected and periods of greater than average windspeed. On dry, sunny days the Burkard sampler collected most conidia between 1300 and 1400 GMT, when windspeed was greatest and relative humidity least, and fewest were collected at night. On wet days there were smaller maxima of conidial catches associated with rainfall.

When simulated raindrops (5 mm diameter) were allowed to fall on infected, sporulating bean leaves, conidia were dispersed up to 90 cm in splash droplets. Although pieces of film placed in the crop collected large numbers of conidia during wet weather, the majority were not in splash droplet traces. Thus it seems that, in crops, most *B. fabae* conidia are dispersed dry by wind, even during periods of rainfall. (Creighton and Fitt)

***Seed-borne viruses of* Vicia faba.** Broad bean stain virus (BBSV) and broad bean true mosaic virus (BBTMV) were detected by enzyme-linked immunosorbent assay (ELISA) in, respectively, 18 (47%) and 12 (32%) of 38 lots of broad bean and field bean seed (ten samples of 25 seeds tested per seed lot). Seed extracts were prepared as for tests by immunospecific electron microscopy (ISEM) (*Rothamsted Report for 1982,* Part 1, 200) and were then diluted (1:1) with phosphate buffered saline containing 0·05% 'Tween 20' and 2% polyvinyl pyrrolidone. No infection with BBSV or BBTMV was found in seedlings grown from seed lots in which little (A_{405} value in ELISA <0·5 after 1 h at 20°C) or no virus was detected in seed extracts, but 0·4–3·7% infection was found in seedlings grown from seed lots in which one or more extracts contained much virus (A_{405} value >1).

***Viruses of* Vicia faba *in China*.** The most common and widespread virus isolated from faba bean crops near Nanjing in 1983 (*Rothamsted Report for 1983,* 125) a strain of broad bean wilt (BBWV), was found to be serologically related to, but distinct from, nasturtium ringspot virus (BBWV serotype I group). The virus caused systemic infection in pea and soybean. The Nanjing virus sedimented as three components with sedimentation coefficients of 62S, 93S and 120S. During equilibrium centrifugation in CsCl the 120S component formed two zones with buoyant densities of 1·409 and 1·464 g cm^{-3}. The buoyant densities of the 62S and 93S components were 1·301 and 1·379 g cm^{-3} respectively.

A virus with filamentous particles *c.* 800 nm long from plants showing a mild yellowish mottle and sometimes yellow veinbanding or reddish ringspots was identified as a strain of turnip mosaic virus. It caused systemic infection in several legume and cruciferous species including pea, peanut, oilseed rape and turnip. Another virus with filamentous particles *c.* 750 nm long from plants showing a mild yellowish mottle was identified as a strain of soybean mosaic virus; it caused systemic infection in cowpea and pea. This is the first time that natural infection of *Vicia faba* with turnip mosaic and soybean mosaic viruses has been reported. (Xu, Cockbain; Woods and S. E. L. Roberts)

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Cereal diseases

Cultivar mixtures. Experiments to examine the effects of interplot interference on the performance of mixtures of spring barley cultivars differing in their susceptibility to powdery mildew (*Erysiphe graminis* f. sp. *hordei*) (*Rothamsted Report for 1982*, Part 1, 192) were continued. In 1983 crop growth was adversely affected by unusually wet weather in spring and this may help to explain why residual variation was large and significant interplot effects undetectable. A mixture of cvs Claret (resistant), Patty and Goldmarker (susceptible) grown in a serially balanced design (where interplot interactions were expected to occur) gave a mean yield which was almost identical (−0·7%) to the mean yield of the component cultivars grown as pure stands in the same experiment. However, in a randomized block experiment in which plots were separated by dummy plots of the mildew-resistant cultivar Atem, the mixture gave a mean yield which was slightly, but not significantly, more (+2·1%) than the mean yield of the components grown as pure stands.

In 1984, the yield data had very much smaller coefficients of variation and there was good evidence that interplot interactions did occur. A mixture of cvs Atem (resistant), Patty and Triumph (susceptible) grown in a serially balanced design, yielded significantly less where next to pure stands of Patty than where next to pure stands of Triumph. These differences in yield may have been, at least partly, due to greater disease development on plants of Patty in the mixture when adjacent to pure stands of Patty than when separated from pure stands of Patty by one of the other cultivars. Inoculum from the very susceptible cultivar Triumph must also have moved between plots but may have been less important in determining the performance of that cultivar in the mixture because mildew was severe on Triumph, in both the pure stands and in the mixture, regardless of neighbours. However, this inoculum may have induced resistance in Patty and hence contributed to the relatively larger yields given by the mixture when grown next to Triumph. This explanation is supported by the significantly greater yields obtained from plots of Patty when next to Triumph than when next to Atem or the mixture. The mean yield of the mixture in the balanced design was 2·8% greater than the mean yield of the component cultivars grown as pure stands but, in a randomized block experiment in which plots were again separated by dummy plots of Atem, the mixture gave a mean yield that was 5·5% greater than the mean of the components. (Jenkyn)

Development of eyespot lesions in wheat. When wheat plants at the two leaf stage were inoculated in growth cabinets with *Pseudocercosporella herpotrichoides* spores impregnated on to filter paper discs, the fungal mycelium penetrated more rapidly through the leaf sheaths at 10°C night/15°C day than at 5°C night/10°C day. However, the death of leaf sheaths was also more rapid. Consequently, the number of living, infected, leaf sheaths was less in the higher temperature regime. Regressions of number of leaf sheaths penetrated and number of dead leaf sheaths on accumulated temperature above 6°C over the 16 weeks of the experiment were both significant ($P<0{\cdot}001$) with positive slopes of 5.5×10^{-4} and 7×10^{-3} respectively.

In 1983–84 the development of eyespot lesions was more rapid than in 1982–83 (*Rothamsted Report for 1983*, 120) perhaps because sowing was on 9 September rather than 2 November. The incidence of severe lesions (14%) was less than in 1981–82 (44%) when spring rainfall was greater.

A generalized linear model was used in an attempt to fit a linear predictor model, based on weather data, to the patterns of eyespot development in the three seasons. The best three-variable model, which accounted for 47·4% of the variance, related the development of eyespot lesions to mean temperature, accumulated temperature and rainfall (lagged by two weeks). The best single variable was accumulated temperature

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but, because it increases uniformly each year, it would not be useful in a predictive model. (Higgins and Fitt)

Joint work on fungicide resistance in eyespot *(Pseudocercosporella herpotrichoides)* populations is reported by Insecticides and Fungicides Department.

Take-all

Control of take-all in winter wheat; further tests. In 1983 the fungicides, nuarimol and benomyl, and the bacteria, *Bacillus cereus* var. *mycoides* and *B. pumilis* (previously referred to as a 'coryneform bacterium'), were tested as soil drenches on small plots on sites with naturally-occurring take-all (*Rothamsted Report for 1983,* 122–123). The infectivity of soil in one site, The Pightle, was assessed after harvest 1983 and, although all plots that had been treated with bacteria were most infectious, the differences amongst treatments were not significant. The experiment was repeated with modifications in 1984: benomyl was omitted, nuarimol was applied in autumn at 1 or 3 kg ha^{-1} with and without a spring application of *B. pumilis,* bacteria were applied in autumn and spring, and *B. pumilis* was also tested alone as a spring application. Take-all ratings in April were 0·93 (the maximum rating is 3) in a third crop on The Pightle, Woburn and 0·64 in a second crop on Long Hoos at Rothamsted. The only significant effects at this time were on disease and were associated with nuarimol, which decreased the take-all rating (mostly by decreasing the proportion of nodal roots infected) at both rates and the percentage of dead shoots at the highest rate at Rothamsted, but increased the percentage of dead shoots at the lowest rate at Woburn. The Long Hoos crop gave four times the yield of the crop on The Pightle, where nuarimol at the high rate increased the number of ears and plot yield. Nuarimol at uneconomic rates has significantly decreased take-all ratings at Rothamsted in two consecutive years and significantly increased yield in two out of four crops.

Although there was more take-all in April 1984, it was less variable than in 1983 and disease variability in small plots (*c.* 35×35 cm) was hardly less than in 1 m^2 areas at Woburn, but was approximately halved at Rothamsted. In both years of these tests small plots in second wheat crops have had less disease variability than larger areas. (Hornby; Henden with Bateman, Insecticides and Fungicides Department, Payne, Statistics Department and Dr R. Campbell, University of Bristol)

Oilseed rape and the take-all fungus. Wheat is often grown after oilseed rape and it has been suggested that this may favour take-all: lesions on rape roots and sometimes more take-all after rape than after other break crops have been reported from Australia (Kollmorgen, Griffiths & Walsgott, *Plant Pathology* (1983) **32,** 73–77).

Forty-eight plants of each of eight rape cultivars were grown in pots of soil from a field in which wheat had been the last crop, with and without amendments of field-grown wheat roots bearing take-all lesions. Discoloured or lesioned rape roots were separated into five caregories, but represented only a very small proportion of all roots. Two out of 30 washed root segments from a small category with take-all like lesions produced cultures of *Gaeumannomyces graminis* var. *tritici,* which were strongly pathogenic on wheat. No such isolates were obtained from the other disease categories, or from 30 surface-sterilized root segments from the same disease category. The take-all fungus does not appear to colonize rape roots readily. (Hornby; Davis)

Assessing soil suppressiveness. It is generally assumed that take-all-decline soils suppress added inoculum, but added inoculum was not conspicuously inhibited in continuous spring barley soils (*Rothamsted Report for 1980,* Part 1, 184). The take-all

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fungus infected similar percentages of roots of wheat plants grown in sand to which had been added, at concentrations of 0·09 or 0·75% (w/w), grains of oats, millet or ryegrass colonized by the fungus. All treatments decreased plant height and weight. Plants exposed to ryegrass inoculum were the tallest and heaviest of the treatments and developed more roots than plants grown in sand with autoclaved ryegrass grains.

Soil from a first wheat crop in a sequence experiment at Rothamsted infected 5% of roots in bioassays and soil from a seventh crop on a neighbouring plot where take-all decline appeared three years earlier, infected 21%. The soil from the first crop had slightly more disease than that from the seventh after incubation for one month with 0·75% added inoculum, but slightly less with 0·09%, and although different inocula produced similar levels of disease at 0·75%, the ryegrass inoculum was most infectious at 0·09%. At the highest concentration autoclaved and untreated ryegrass inocula produced plants that were heavier and greener than those exposed to other inocula. Whilst there is no standard procedure for the added inoculum technique, it should be noted that in these experiments (a) there were no marked differences in disease levels after adding inoculum to soils that had grown a first or a seventh wheat crop; (b) interpretation was complicated by endemic inoculum and (c) ryegrass inocula produced better plants than other inocula despite similar percentages of infected roots. (Hornby; Davis)

Barley yellow dwarf virus (BYDV)

Forecasting 1983–84. The forecast last year for infection of autumn-sown cereals was that the risk from migrant aphids, as measured by the Infectivity Index (II), was low. This forecast was largely fulfilled although there were occasional, severely affected crops which seemed to be associated with the carry-over of aphids on virus-infected volunteers or grassy stubbles. In some areas, especially the North Midlands and South Yorkshire, widespread and damaging infection by BYDV was reported to be associated with *Sitobion avenae*. The extent of this infection was not reflected in infectivity measurements at Leeds but resiting the infectivity trap in 1984 appears to have resulted in catches that better represent the incidence of aphids on crops.

Autumn 1984. Infectivity was measured at eight of the same sites as in 1983. The trap at Leeds was resited at Headley Hall and a new site was at Wolverhampton (ADAS). Results are incomplete but the Infectivity Index at most sites is about the same or larger than last year. The largest indices have been recorded at Wolverhampton (II=348) and Shardlow (II=208). The predominant aphids caught at all sites have been *Rhopalosiphum* spp., but at Headley Hall the ratio of *Rhopalosiphum* spp./*Sitobion* spp. was 3:2 compared with ratios of greater than 10:1 elsewhere. Infective *S. avenae* were caught after 1 September at Headley Hall, Long Ashton, Rothamsted and Wolverhampton. Based on these figures it seems that the risk from BYDV introduced by migrant vectors is similar to or slightly greater than last year in Southern Britain but in the Midlands and North is greater than in 1983. (Plumb; Gutteridge, Grossett, Lennon and ADAS)

Infectivity—spring and summer. Infective migrant aphids were not caught until 11 June (*R. padi* and *S. avenae*). The first infective *Metopolophium festucae* was caught on 22 June and *M. dirhodum* on 2 July. Virus introduced on these dates to any but the latest spring-sown crops was not expected to be serious and virus infection of spring barley was slight. Aphids migrating from ripening cereals were most numerous in July when 5·2% were infective. (Plumb; R. A. Gutteridge, Hubbard and Lennon)

Brome mosaic virus (BMV). BMV infection of grasses is widespread in Britain but has never been associated with a damaging disease. However, reports that the virus is

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transmitted by cereal feeding aphids as well as mechanically prompted an investigation of the potential BMV has for damaging cereals in Britain. One hundred plants each of spring barley cv. Triumph and spring wheat cv. Minaret were manually inoculated with BMV a month after sowing just before tillering. On barley, symptoms appeared 11 days after inoculation; plants showed a diffuse mosaic, were yellow and stunted. At harvest all components of yield were significantly decreased by infection, fertile shoots by 10%, height by 16%, grains/ear by 7%, 1000 grain weight by 9% and yield by 25%.

Symptoms on wheat were not as severe as on barley. Fertile shoots and grains per ear were unaffected by infection and plant height was increased by 7%. However, 1000 grain weight was decreased by 12% and yield by 17%. (Hubbard)

Barley yellow mosaic virus (BaYMV). BaYMV, transmitted by the soil-borne fungus *Polymyxa graminis,* continues to increase in importance in all the main winter barley growing areas of Great Britain. No fungicide is known to control the vector and control strategies have centred on growing cultivars resistant to the virus and on minimizing spread of infested soil to new sites.

In screening tests using a mechanical inoculation procedure, most winter barley cultivars were susceptible and produced obvious symptoms 10–14 days after inoculation but some, e.g. Sonja, Igri, produced fainter symptoms which appeared in two to three weeks. Only one of the cultivars tested (Athene) appeared to be immune.

The virus affects only barley (and in the field only autumn-sown crops show symptoms) but *P. graminis* is reported from other cereals and grass weeds. However, attempts to inoculate an isolate from barley on to wheat and oats were unsuccessful. On two sites where barley roots were infected with *P. graminis,* wheat plants on the same field were apparently uninfected. These results suggest that there may be fungal races specialized to different host species. A viruliferous isolate of the vector was, however, grown on roots of the virus-immune winter barley cv. Athene. Zoospores and cystosori (resting spores) from Athene were used to establish fresh cultures on the BaYMV-susceptible cv. Maris Otter but very few plants became infected compared with those inoculated with the vector from a BaYMV-susceptible host. If the growth of some barley cultivars decreased the virus content of a vector population this would have important implications for disease control. (Adams)

Potato diseases

Sampling potatoes for the incidence of inoculum and tuber diseases. Experiments in 1980–82 were designed to quantify the sample sizes needed for disease assessments. Between three and six seed stocks of cv. Pentland Crown were planted each year and seed tubers, stem bases and progeny tubers from the growing crop and tubers at harvest and after storage were sampled intensively using standard procedures to estimate inoculum levels or disease incidence for skin spot *(Polyscytalum pustulans),* silver scurf *(Helminthosporium solani),* black scurf *(Rhizoctonia solani),* gangrene (*Phoma exigua* var. *foveata*) and bacterial soft rot *(Erwinia carotovora).*

The data were used to calculate sample variances after transformation of percentage values to logits. A variance of 0·010 was selected as giving acceptable accuracy for most purposes. To achieve such a value, five to ten 50-tuber samples of seed tubers or 15–20 samples of a harvested or stored crop were required for assessing disease symptoms or for assessing gangrene potential by a standard damage test. The eye plug test for assessing inoculum of blemishing diseases needed 15- or 20-tuber samples and five such samples of seed or about ten from the growing crop were usually adequate. Variances were nearly always greater for black scurf assessments than for other blemishing diseases and the disease was more patchily distributed in the field.

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The results have demonstrated the difficulty of obtaining an adequate sample, especially from very large fields, but will assist in planning future experiments and surveys. (Adams, Hide and Lapwood)

Forecasting storage diseases. In 1983 data were first collected on the effects of weather, soil type etc. on the development of storage diseases by planting seed tubers from five Désirée seed stocks on three Experimental Husbandry Farms (Arthur Rickwood, AR; High Mowthorpe, HM; Terrington, TT) and at Rothamsted (RES). Crops were sampled in August and produce from all crops was stored at Rothamsted. Stem canker and black scurf were most common at AR and RES and skin spot at HM; diseases were most prevalent on crops from seed with much disease. Most silver scurf developed on tubers from HM and RES and usually on crops from seed with least disease. Almost 90% of tubers from all stocks grown at AR had black dot; on other farms the disease was scarce but its incidence was related to the severity of seed tuber infection. Gangrene was prevalent on tubers from a severely affected seed stock grown at RES, and also at RES and HM on tubers from seed inoculated with *Phoma exigua* var. *foveata*. Blackleg was found only at AR (irrigated site) and in August, after incubation under water, most soft root developed on tubers from AR and TT, especially from a seed stock that had shown a large amount of rotting in tests before planting. At harvest, tubers from AR developed least soft rot.

Although these results confirmed the importance of seed tuber-borne inoculum, they also showed that differences in disease incidence were usually larger between sites than between seed stocks, and that these differences were already present 9–11 weeks after planting (August sample). Tubers from TT had least skin spot, black scurf, silver scurf and black dot, suggesting a disease-suppressive effect on that farm. (Hide; Adams, Lapwood and Read)

Blackleg (*Erwinia carotovora* ssp. *atroseptica*)

Susceptibility of cultivars. Experiments with the National Institute of Agricultural Botany, Cambridge, have established a procedure for assessing the field susceptibility of cultivars to blackleg disease (see *Rothamsted Report for 1982,* Part 1, 205). Six cultivars were assessed in field trials in 1982, 12 in 1983 and 18 in 1984. Simplification and speeding of the scoring procedures now allows the inclusion of many more. From these experiments it should soon be possible to publish a susceptibility score for blackleg disease in the NIAB Recommended List. (Lapwood; Read with Mr P. T. Gans, NIAB, Cambridge)

A simple, reliable growth room method for assessing susceptibility to blackleg disease on new cultivars and breeders' clones would be an invaluable supplement to field trials where weather and disease severity may differ between seasons. To meet this need a tuber seed piece or 'dome' method has been developed. The seed tuber is cut across the middle at a standard distance, usually 5 cm from the apical end. A wet pad impregnated with bacteria from a standardized inoculum is applied to the cut surface and kept in place by 'clingfilm'. Since 1982 up to 24 different cultivars have been tested each year in this way in the field and clear and consistent differences have been shown. Recently tests with a few cultivars have shown that differences can be reproduced in growth rooms. (Lapwood; Read)

Potato virus diseases at Rothamsted. When counts were made in early July, plots planted with Désirée seed grown at Rothamsted in 1983 had 1% infection with potato virus Y (PVY) and 0·3% potato leaf roll virus. Pentland Crown was free from these

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diseases but plots planted with Foundation Seed King Edward had 0·2% PVY. Aphids *(M. persicae)* were relatively few and only limited spread of PVY was detected, even in a King Edward crop grown to provide seed for 1985 in which 1% tuber-borne PVY infection was present as a source of infection. (Govier)

Primarily-infected potato plants as sources of potato virus Y (PVY) in crops. It is unclear whether potato plants infected with PVY when young can be sources of virus for further current-season spread. The strain of virus may be important as mature plant resistance may develop against PVY^O but not PVY^N. In mid-June, plants of cvs Record and King Edward were inoculated with either PVY^O or PVY^N. About 65% of tubers harvested from plants next to those inoculated were infected compared with 22% around uninoculated plants. (Katis; Gibson)

Vectors of potato virus Y. PVY is transmitted in the non-persistent manner by aphids and is spread by potato colonizing and itinerant species. Of aphids trapped on a net downwind of a plot of PVY-infected potatoes *c.* 2% out of 7000 transmitted PVY to tobacco test plants. Twenty species were found to be infective and included *Metopolophium festucae, Myzaphis rosarum, Myzus ligustri, Myzus myosotidis* and *Sitobion fragariae*; all are newly-reported vectors of PVY. In June, *Brachycaudus helichrysi* transmitted most frequently. In mid-July *Myzus persicae* and from mid-July to mid-August *Phorodon humuli* and various *Aphis* species were most frequently infective. A comparison of infective vectors over several years should enable Insect Survey suction trap catches to be interpreted as a vector index. (Katis; Gibson with Harrington, Entomology)

Aphid transmission of two strains of potato virus Y (PVY) from doubly infected plants. Of *M. persicae* allowed access to a doubly infected tobacco plant for 2·5 min, 19% transmitted PVY^O, 12% transmitted PVY^N and 20% transmitted both PVY^O and PVY^N; 20% of *M. persicae* allowed only a single acquisition probe in a doubly infected plant also transmitted both strains. Thus there was no indication that the duration of acquisition probes influenced which viruses were transmitted.

The ability of *M. euphorbiae* and *R. padi,* to transmit both strains from doubly infected plants was compared with that of *M. persicae. R. padi* was tested in groups of 20 but *M. euphorbiae* and *M. persicae* were tested singly. Sixty-nine per cent of *M. persicae,* and 9% of *M. euphorbiae* transmitted either PVY^O or PVY^N and, of those that transmitted, 25% and 2·4% respectively transmitted both strains. For the groups of 20 *R. padi* the corresponding transmission rates were 26% and 1·7%. This suggests that the less efficient vectors, *R. padi* and *M. euphorbiae,* inoculated fewer virus particles than *M. persicae.* (Katis; Gibson)

Diseases of tropical crops

An apparently new virus disease of melons was found in crops from the People's Democratic Republic of Yemen. The virus is serologically related to some tymoviruses but not to mild cucumber mosaic virus, the only other tymovirus known to infect cucurbits. A virus morphologically similar to tombusviruses was found in sorghum from India and was mechanically transmissible to sugarcane.

The non-capsid protein accumulated in plants infected with maize stripe virus or maize chlorotic stripe virus was shown to be similar in size to the pathogenesis-related proteins present in TMV-infected tobacco. However, as it is basic not acidic and has a very different amino acid composition its function is unlikely to be the same as that of the PR proteins.

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Mycoplasma-like organisms were found in the phloem sieve elements of *Vicia faba* from the Sudan showing phyllody and in 8 of 20 clove trees affected by sudden death disease in Zanzibar and Pemba. The results suggest that for both diseases mycoplasma may be the causal agent. (Jones; Carpenter and Cockbain with Drs A. Dabek and P. Martin, Zanzibar and Dr E. Kondaiah, India)

Viruses and virus diseases

Beet mild yellowing virus (BMYV). Immunodiffusion tests with an antiserum prepared to an American strain of beet western yellows virus (BWYV) confirmed the close relationship between this virus and BMYV indicated by earlier tests using ISEM and ELISA. A virus isolated from oilseed rape reacted strongly in ELISA with antisera to both BMYV and BWYV. (Govier)

Beet mosaic virus (BMV). Published methods for purifying BMV were ineffective for an isolate multiplied in *N. clevelandii*. However, 0·5 M borate buffer at pH 8 containing 0·05 M EDTA was used successfully for the initial extraction. The molarity, pH and presence of EDTA were all important for good recovery of virus although phosphate but not tris can be substituted for borate. When the preparation was purified by cycles of differential centrifugation the solubility of the virus in buffer decreased, so that after the third centrifugation pellets were dispersed best in water. (Carpenter)

Virus charge and aphid transmission. Morales (*Abstracts of Meeting on Plant Disease Epidemiology, Oxford 1981,* 100) found an inverse correlation between efficiency of aphid transmission and anionic electrophoretic mobility of five bean yellow mosaic virus (BYMV) isolates. We found no similar correlation for some other potyviruses. Electrophoretic mobility as anions for three viruses was in order of increasing mobility beet mosaic virus (BMV), PVY^O, PVY^N; whereas ranking for increasing efficiency of transmission in competitive tests was PVY^O, BMV, PVY^N (*Rothamsted Report for 1983,* 119). Charge interactions in the transmission process are not ruled out but must be more subtle than the total charge on virus particles. (Carpenter)

Work on disease resistance is reported in the Molecular Sciences Division.

Aerobiology

Spore dispersal gradients. Empirical models for describing gradients of spore concentration, spore deposition or disease with distance away from sources of inoculum were compared, using published and experimental data. Of 124 gradients resulting from dispersal of dry air-borne spores by wind, 59 were fitted best by power law models and 65 by exponential models. Fifteen deposition gradients for spores dispersed in splash droplets were fitted best by exponential models. Such models were used to interpret results of field experiments to investigate the effects of mixtures of spring barley cultivars on dispersal of *Rhynchosporium secalis* spores and spread of leaf blotch. (Fitt and Creighton with McCartney, Macdonald and Quayle, Physiology and Environmental Physics Department)

Further joint work is given in the report of the Physiology and Environmental Physics Department.

Airborne microorganisms associated with domestic refuse disposal. The handling and disposal of domestic refuse may expose workers to airborne microorganisms which could pose a potential health hazard. Regular air sampling at 12 transfer stations and landfill sites in the London area, three in West Sussex and two in South Yorkshire compares

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methods of waste handling and geographical sources and measures seasonal variations in airborne and microorganisms.

Initial results indicate concentrations of airborne microorganisms two to 100 times those upwind of the source depending on the location and type of operation. Airborne bacteria that grow at 37°C numbered up to $3{\cdot}6\times10^5\,m^{-3}$ air and those at 25°C, $5{\cdot}2\times10^5\,m^{-3}$ air while fungi numbered $8{\cdot}3\times10^4$ and $1{\cdot}3\times10^6\,m^{-3}$ air respectively at these temperatures. Greatest numbers of microorganisms were sampled when refuse was removed from storage bunkers by grab-crane. Predominant fungi were species of *Penicillium* and *Aspergillus*. Fluorescent *Pseudomonads* were the predominant Gram negative bacteria but the *Klebsiella/Enterobacter* group were also numerous. Most Gram positive bacteria were *Bacillus* spp. although cocci were also common. (Lacey and Crook)

Biodeterioration

***Fusarium* contamination of cereal grains.** *F. poae* and *F. tricinctum* were the predominant *Fusarium* spp. isolated from wheat ears during ripening. *F. poae* was present in small numbers from anthesis and *F. tricinctum* was first isolated four weeks later (Zadoks growth stage 85). Both species increased rapidly following rain in early August as the crop ripened to Zadoks growth stage 92. Final counts were 46000 *F. poae* and 20000 *F. tricinctum* propagules g^{-1} fresh wt. *F. culmorum* and *F. avenaceum* were found in small numbers on few occasions in contrast to 1982 and 1983. (Lacey)

Tests of chemicals to inhibit respiration of cut grass. Test chemicals were sprayed on to freshly cut grass using a hand held spray gun and 0·1–0·4 g a.i. applied in 2 ml water to 100 g hay. The treated grass was placed in a layer between plastic mesh inside clear perspex tubes, 1 m long×15 cm diameter, which were then sealed at both ends. A controlled flow of air dried over silica gel from which CO_2 had been removed with soda lime was passed over the grass and into a CO_2 analyser equipped with a chart recorder. 'AG 58' (BP Chemicals), applied at 0·4%, was most effective in preventing moulding and much better than formic acid, commonly used as a desiccant for silage. 'AG 58' decreased respiration by 55–60%, compared with untreated grass, and allowed drying of a leafy crop from about 75% water content to 40% in 2–2·5 days. Formic acid inhibited respiration by only 25% and allowed drying to only 49% in the same period. Applied at 0·3–0·4%, 'AG 58' inhibited yeasts and filamentous fungi initially but they partially recovered after three to five days. Chemicals tested tended to be more effective on young leafy crops than on older stemmy crops. 'AG 58' was also more effective on conditioned than on unconditioned crops. (Lacey: Magan)

Staff and visiting workers

I. Macfarlane and G. H. King retired after 39 years and 33 years respectively. G. Muthyalu took early retirement, Maureen Lacey transferred to the Physiology and Environmental Physics Department and Janis Spokes resigned. M. Weller left on completion of his temporary appointment and N. Magan left to take up a post at Glasshouse Crops Research Institute. Sally Higgins, Susan Marriott, N. Katis and B. Crook were awarded Ph.Ds and Maryse Chabrol returned to France after completing her studies on the effects of soil sterilants on maize. Valerie Church transferred from Insecticides and Fungicides Department and June Newman joined the Department as a part-time specialist typist (shared with Nematology). In addition 14 visiting workers and students, Xu Zhi-gang and Guan Shi-he (People's Republic of China), Eliane Dumas

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(France), J. Chanda (India), U. Palmgren and G. Ström (Sweden), K. House (Australia), Helen Gunn (AFRC Bursar), J. Geertsema (Netherlands), Rodiah Balfas (Indonesia), C. Davis, K. Grossett, Vanessa Hubbard and Deirdre Rose contributed to the Department's work. B. D. L. Fitt was promoted.

D. Hornby organized a very successful Anglo-French Workshop at Rothamsted on 'Methods for studying biological control of diseases caused by soil-borne plant pathogens' and continued as Chairman of the International Society for Plant Pathology's (ISPP) Committee on Soil-borne Plant Pathogens. J. Lacey is Chairman of the ISPP Committee on Mycotoxicology. Members of the Department contributed as session organizers as well as by papers, posters and chairmen at scientific meetings and as demonstrators at agricultural events.

Maryse Chabrol and D. Hornby presented a poster at a workshop in Versailles, D. Lapwood contributed to the International Conference on Potato Blackleg Disease in Edinburgh, C. J. Rawlinson was a UK delegate to an EEC workshop on Rapeseed in Copenhagen, R. T. Plumb, supported by The British Council and the Volcani Institute, visited Israel and attended a workshop on Epidemiology and Diagnosis of Plant Viruses, and attended the 4th Conference of the European Working Group on Virus Diseases of Gramineae. R. W. Gibson visited the Volcani Institute, Israel, R. H. Turner attended the Scanning Electron Microscope Symposium in Philadelphia and R. F. White the Leipzig Biotechnology Symposium at which he was Chairman of the session on resistance inducers. J. Lacey visited Germany at the request of the Ministry of Defence.

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