ROTHAMSTED RESEARCH

Rothamsted Experimental Station Report for 1986

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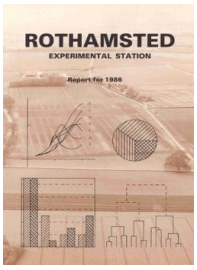

Crop Protection Division

T. Lewis

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INTRODUCTION

For the third year in succession enforced staff reductions have interrupted several important programmes, yet the dedication and enthusiasm of staff remains commendably high. The flow of outside funding has enabled some of these losses to be made good on a short-term basis, but further erosion of core funds would deplete some projects beyond the point where they can continue to attract external interest.

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During the year many members of the Division have been actively involved in stimulating closer links with other AFRC Institutes and organizations concerned with the compatability of farming and wildlife. Direct funding from public and industrial sources has been forthcoming for this type of work, enabling further emphasis to be placed on the biological control of invertebrate pests and fungal pathogens, and on the development of novel chemical methods for limiting pest and disease damage. Of particular note is the demonstration, for the first time, of the restriction of a plant virus by an environmentally harmless antifeedant applied to deter its aphid vector. Work on the biological control of nematodes has also gathered momentum as the team of six new appointees working on this project has been recruited and settled in. Examination has begun of the long run of data on moth abundance and distribution collected by the Insect Survey, to see if it can be used as an indicator of environmental change brought about by changing agricultural practices or land use. Possible biological control agents for take-all, the most intractable disease of cereals, have been compared and evaluated. These are but a few examples of a large body of work, about half the Division's effort, relevant to limiting the use of pesticides, and the development of resistance to them, thereby encouraging a more abundant and diverse wildlife fauna on farmland.

Divisional staff have played a full part in the wider Station effort to explore prospects for alternative arable crops, including an assessment of possible pests and diseases of sunflowers and lupins; pollination studies of these crops are also planned. On the more traditional crop of potatoes, encouraging new approaches to the control of non-persistent viruses and storage diseases have been successful.

Roger Plumb was awarded the Royal Agricultural Society of England's Research Medal for his work on Barley Yellow Dwarf Virus, which has led to the development of a rational basis for pesticide use to control this disease, based on economically and environmentally acceptable criteria. This is an indication of the high regard in which Rothamsted's agricultural science and scientists are still held. Long may such excellence, and the public recognition of it, continue!

ENTOMOLOGY DEPARTMENT

Most of the Department's work is now related to minimizing pesticide use and developing alternative or supplementary methods of pest control. Studies on monitoring and forecasting of various pest populations to assess the need for control continue as a major activity, supported by basic work on insect population dynamics and behaviour. Looking ahead to possible alternative crops for British agriculture, preliminary observations on potential pests of lupins and sunflowers have begun. Despite further losses of permanent staff, several sources of public and industrial short-term funding have been gained, allowing temporary staff to be appointed to analyse the large database on moth abundance and distribution in relation to changing land use, to investigate the role of natural enemies in controlling cereal aphid populations, and to elucidate the involvement of insects in triggering automatic fire alarm systems.

Aphid studies

The main emphasis of research within the Insect Survey has been to improve interpretation of suction trap samples, to provide better up-to-date information to the agricultural industry and to understand insect movement. Some of the topics studied are detailed below.

Monitoring. The information on pest aphids derived from the routine suction trap monitoring of migrant aphids was distributed to the agricultural industry by mail and by

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viewdata. Interested parties received the *Aphid Bulletin* and the *Aphid Commentary* by mail (*Rothamsted Report for 1983*, 89). The agricultural interpretations of suction trap data in the *Aphid Commentary* formed an *Aphid Warning* section, updated weekly, within the *Rothamsted Farming Service* viewdata package available on Prestel Farmlink and Agviser, the two national agricultural viewdata systems. (Tatchell; Dupuch, French, Mitchell, S. J. Parker, D. K. Riley and Taylor)

The data gathered from the suction trap samples, together with that from the Insect Survey light trap network, forms a large and ever increasing database of over 50 Mbytes of information. It is therefore essential to have a very efficient computer database system. Currently two databases are operated, a microcomputer based system for quick data entry and analysis of aphid data to provide up-to-date information, and a tape based system on the Computing Centre's VAX 11/785 for the historical database used for research.

An online disc based system for the historical database using the 1032 database management system is now being implemented. All existing data have been loaded, a data management system developed and software for users is under development. The completion of this system will improve access to the Insect Survey's data for all users and enable better interpretation of the historical and current databases. (Woiwod; Wong with Summerfield, RESCU and Clarke, Statistics)

Cereal aphids in summer. The Rothamsted Insect Survey Cereal Aphid Monitoring Scheme (RISCAMS) continued to monitor field to field variation in aphid numbers and to improve interpretation of suction trap samples. Twenty fields around each of the suction traps at Kirton, Broom's Barn, Rothamsted and Littlehampton were sampled. As in 1985 (*Rothamsted Report for 1985*, 86) weekly summer samples assessed aphid abundance and natural enemy acitivity in each area.

The winter of 1985/86 was very cold, few aphids survived on crops around Rothamsted and crop development was retarded, as in 1984/85. Very few aphids were found in crops in any of the four areas when sampling began between the end of May to mid-June. Numbers of immigrant cereal aphids remained low throughout June so that populations on crops increased slowly and all were very much below one per shoot, thus below Agricultural Development and Advisory Service (ADAS) thresholds for control, at flowering. Populations increased more rapidly during July and in one field *Metopolophium dirhodum* reached more than 50 per shoot. Peak emigration from crops occurred during early to mid-August and numbers were relatively higher for *M. dirhodum* and *Sitobion avenae* at Broom's Barn, Rothamsted and Kirton compared with Littlehampton.

Populations were generally low so there was little variation between fields. However in a few fields large populations did develop, possibly because high nitrogen and fungicide input kept the crops greener, and hence more susceptible to aphids, for longer. Natural enemy populations also developed later although syrphids, especially in the Littlehampton area, did become numerous later on. Aphid-specific predators were relatively scarce at all times in the Kirton area. The consequences of these apparent regional variations in natural enemies are poorly understood but are one of the major objectives of RISCAMS. (Carter)

Cereal aphids in autumn. The bird-cherry aphid, *Rhopalosiphum padi*, is considered as the principle vector of barley yellow dwarf virus (BYDV). In autumn three alate morphs are present in suction trap samples. Males can be identified readily, but the alate virginoparae and gynoparae are morphologically indistinguishable. The virginoparae colonize and reproduce on cereals, while the gynoparae fly in search of the primary host plant, *Prunus padus*. In an attempt to separate these two morphs biologically aphids were trapped alive at 1·5 m and 12·2 m in suction traps. Samples were taken twice daily from August to early November and the 1500 or so female *R. padi* trapped were tested individually by giving them

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a choice of barley or *P. padus*. After 48 h the female morph was determined by observing the host plant selected, and counting and identifying the morph of the offspring. At the same time *P. padus* trees and sequentially-sown plots of barley were monitored for the appearance of alate aphids.

Results indicate that in 1986 at Rothamsted the first gynoparae appeared in mid-August and by mid-September the alate female *R. padi* were exclusively gynoparae. In October a small percentage of the population was again virginoparae. The change in morph structure also coincided with a change in the density-height profile of this aphid with more virginoparae being recorded at 1·5 m but more gynoparae at 12·2 m. The consequences of these findings for crop infestations and infection by BYDV will be studied further. (Tatchell, Carter)

Aphid vectors of potato virus Y (PVY). The potential of various aphid species as vectors of potato virus Y was again tested in the field by trapping alate aphids on a net downwind of a crop totally infected with PVY and confining the aphids overnight on tobacco plants to test individuals for infectivity. Sixteen species were found to be vectors, including *Aphis sambuci* which is reported as a vector for the first time. Of transmissions, 21% were by *Myzus persicae* and 20% by *Aphis fabae*.

An assessment of the risk of PVY in crops being considered for use as home-saved seed in southern Britain is now issued each August on the Prestel Farmlink and Agviser viewdata services. The assessment is based on numbers of *M. persicae* and *Brachycaudus helichrysi* caught in suction traps in June and July. A large flight of *M. persicae* occurred in the east at the end of July after a season of low aphid numbers up to that time. The PVY risk assessment for eastern England for planting home-saved seed in 1987 is moderate for susceptible cultivars and low for resistant cultivars and for all cultivars in other regions. (Harrington, with Gibson and Govier, Plant Pathology)

Improving suction trap design. It has proved difficult to maintain the structure of the 12·2 m suction traps which have been built largely of plywood since their original design in the early 1960s. A new aluminium type has been developed and tested at Rothamsted. The centrifugal fans have been coated with plastic to prevent the build-up of corrosion and pollutants which are thought to have impaired performance over past years. These improvements will be incorporated in the Institut National de la Recherche Agronomique (INRA) traps to ensure standardized performance.

The calibration of the traps has been difficult partly due to a lack of suitable instrumentation. An integrating, total head probe is being developed which can be located in the pipe to give digital readout of airflow and an analogue signal to provide hard copy. With this device it should be possible to adjust all traps to give identical performance. (Macaulay)

Radar studies. The Rothamsted Insect Survey Radar (RISR), designed to detect airborne insects at heights up to 250 m, was developed further. Research over the past year has concentrated on measuring and improving the performance of the conical scan radar system, which is now housed in a transportable air-conditioned trailer and is undergoing field trials.

Significant advances in the theoretical aspects of the signal processing required to extract the necessary information from insect targets moving rapidly through the radar beams has been made and a number of new signal processing algorithms have been programmed into the radar computer and are being assessed.

Insect radar echoes recorded during the summer are being analysed to determine precisely those characteristics that can be used for categorizing and identifying different insect species. This work is being done in parallel with a New Initiative funded project to determine, in the laboratory, the microwave scattering properties of small insects. The results are

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being compared with the theoretical predictions for dielectric spheroids and ellipsoids and with the measurements taken by the radar itself. The aim is to develop a 'Radar Taxonomy' for pest insects.

A future network of radars is still planned for the 1990s, and with its introduction should come a significant improvement in the Insect Survey's ability to provide accurate up-to-the-minute information on the airborne insect pest situation throughout the UK. (Bent, Cuminetti; Prior with Dr J. Riley, Tropical Development and Research Institute)

Population genetics. During the summer large collections of both pest and non-pest aphids were made from host plants, mainly in south-east England, as part of a long-term study of spatial and temporal genetic variability (*Rothamsted Report for 1983*, Part 1, 90; *Rothamsted Report for 1985*, 95–96). The species currently under investigation include: the blackberry-grain aphid (*Sitobion fragariae*), collected from its main primary and secondary hosts—blackberry (*Rubus fruticosus*) and cocksfoot grass (*Dactylis glomerata*), the bird-cherry aphid (*Rhopalosiphum padi*) collected from the primary host, bird cherry (*Prunus padus*), the rose-grain aphid (*Metopolophium dirhodum*), collected from wheat, the damson-hop aphid (*Phorodon humuli*), mainly from the primary hosts *Prunus spinosa* and *P. domestica* and the Sycamore aphid (*Drepanosiphum platanoides*). In the case of *R. padi*, collections were also made in Bristol and southern Scotland, and for *P. humuli*, in Kent, Herefordshire and around Rothamsted.

Preliminary conclusions for *R. padi* are that because allele frequencies at two polymorphic enzyme loci (glutamate oxaloacetate transaminase (GOT) and sorbitol dehydrogenase (SORDH)) out of 13 surveyed are spatially similar throughout southern England, there is probably significant gene flow between populations in this region. This view concurs with the fact that *R. padi* is caught frequently in 12·2 m suction traps and supports the belief that it is a highly migratory species.

The allele frequency results for *S. fragariae*, assessed only at a single locus (GOT) (*Rothamsted Report for 1985*, 95–96), support previous conclusions that there are local populations with stable gene-genotype frequencies over at least 4–5 years and which, in some cases, show little reciprocal gene flow over relatively short distances (around 1–50 km). This apparent genetic isolation may in turn be associated with the sparse catches of this species in suction trap catches, and its less migratory habits. (Loxdale; Brookes)

Entomogenous fungi for control. Work on the production, formulation and testing of the entomogenous fungus *Erynia neoaphidis* for aphid control continued.

To provide material of *E. neoaphidis* for field testing and for provisional work on the formulation and storage of the product, the fungus was grown in a liquid medium of yeast extract and glucose, supplemented with cows milk. Mycelium, comprising branched hyphae up to about 500 μm in length, produced in this medium was filtered leaving a mat approximately 1 mm thick on the filter paper. This was dried gently for 24 h. After two weeks storage at 10°C and 20% RH the number of conidia produced per unit area of the surface of the remoistened mat was comparable with that produced soon after drying, but after four weeks the number had approximately halved and few conidia were produced at all after 12 weeks.

The conidia from mats stored for seven weeks were as infective as those from freshly-killed aphids, regarded as the most virulent conidia that can be produced, but the target aphids inoculated with the conidia from stored mats died after five days, one day later than those inoculated with conidia from aphids. Mycelial mats that were dried rapidly (in <4 h) in a stream of moving air produced few or no conidia after re-moistening, demonstrating that the drying process is critical to the survival of the mycelium. Attempts to increase the survival period by adding a range of hexose and disaccharide sugars and sugar alcohols to the

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cultures shortly before the fungus was harvested, failed. Also, milling the dried produce through a 1 mm sieve greatly diminished the ability of the material to produce conidia.

Further investigations are needed to identify the drying regimes that will best preserve the viable mycelium and additives that will improve its survival. Consideration must also be given to the preparation of the product as granules or in suspension, suitable for distributing in the field.

An application of a suspension of the unformulated mycelium from shake flasks was made to experimental plots on the evening of 24 June to investigate the persistence of infectivity of the material in the crop. Half the treated plots were covered with metallized polythene during the night and early morning following application. This material ensured moist conditions, while the highly reflective surface kept the crop relatively cool in sunlight. Samples of leaflets were taken 16 h after application and confined for 24 h with healthy pea aphids, *Acyrthosiphon pisum*. The test aphids were then transferred to growing broad bean seedlings and monitored for the number infected.

A sample of 18 leaflets from treated plots caused infection in two instances, both from covered plots. No aphids confined with leaflets from untreated plots were infected. Samples were also taken from adjacent plots treated with the triturated bodies of fungus-killed aphids. Again 18 leaflets were sampled; in seven out of nine instances aphids on leaflets from covered plots became infected, and in five out of nine instances aphids from uncovered plots succumbed. These results suggest that the activity of the fungus produced *in vitro* persists on foliage for a shorter time than the fungus produced in aphids and emphasises the need for a protective formulation.

As demonstrated in 1986, trials with *E. neoaphidis* in the field are sometimes prevented by a failure of the pest population to establish. To overcome this a new series of experiments is being devised in which the fungus will be tested against aphid populations on plots of plants in constant environment rooms. (Wilding, Sherlock; Brobyn, Mardell, Seibt)

A virus from *Sitobion avenae*. Aphid numbers in one culture of *S. avenae* which had been maintained in a glasshouse for over two years, increased much more slowly after sub-culturing in comparison with other cultures of the same insect. Pooled extracts of a small sample of dead aphids from this culture contained many 30 nm virus particles when examined by electron microscopy. Purified virus failed to react with antiserum to barley yellow dwarf virus (BYDV), the principal plant virus transmitted by *S. avenae*, and no reaction was obtained with antiserum to a similar virus isolated from the oat bird-cherry aphid, *R. padi*. Purified particles had a sedimentation coefficient ($S_{20,w}$) of 160 s, a buoyant density of 1·37 g/ml in CsCl and one major capsid protein. Preliminary work suggests that the virus does not multiply in barley, oats, ryegrass or wheat and that it is not transmitted between *S. avenae* individuals feeding on the same plant. The effect of the virus on the longevity and fecundity of *S. avenae* and its occurrence in field populations of aphids are currently under investigation. (Allen and Ball)

Aphid parasitoids. Studies of the ability of the aphid parasitoids *Aphidius ervi* and *Aphidius rhopalosiphi* to switch from one host species to another were extended from the laboratory into the field. Trials using parasitoids from laboratory cultures indicated that individuals reared on one host may be reluctant to switch to another host, their performance being measured in terms of mummy formation (*Rothamsted Report for 1985*, 96). This reluctance would obviously reduce the effectiveness of using alternative hosts to enhance parasitoid activity in field crops. However, small laboratory cultures of Hymenoptera tend to have low genetic variability due to genetic drift and this may have influenced previous results.

A. rhopalosiphi collected from field populations of the rose-grain aphid, *Metopolophium dirhodum*, performed equally well when switched to the grain aphid, *Sitobion avenae*, as

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when left on their original host. This did not agree with the results obtained using laboratory reared parasitoids, whose performance was greatly reduced when switched from *M. dirhodum* to *S. avenae*. This supports our suspicion that the laboratory population had been inadvertently selected genetically, resulting in a strain which performed exceptionally well on *M. dirhodum* but badly on *S. avenae*. These observations suggest that the performance of mass-reared parasitoids may be adversely affected if the host on which they are reared is different from that which they are required to control after release. Also, there may be some potential for the selective breeding of parasitoids to increase their effectiveness against a particular host.

A. ervi reared on pea aphids, *Acyrthosiphon pisum*, in the laboratory performed very badly when switched to nettle aphids, *Microlophium carnosum*, and in this case similar results were obtained when field-collected parasitoids were tested. Therefore this species, which attacks aphids feeding on very different host plants, seems to display a genuine reluctance to switch between its hosts. (Powell; A. F. Wright)

Encouraging polyphagous predators. Work continued on ways of increasing natural enemies of aphids in winter wheat. Pitfall trapping suggested that epigeal, predatory beetles (Carabidae and Staphylinidae) may be encouraged by the addition of organic matter to the soil. The organic matter increased populations of small soil invertebrates which are potential food items for the beetles. However, pitfall captures are difficult to interpret since they are a function of both beetle abundance and activity. Increased food supply in plots treated with organic matter may lead to increased captures of beetles because more beetles congregate in those plots. Conversely, increased food availability will reduce searching activity resulting in fewer beetles being caught.

Because of this difficulty in interpreting increases in pitfall captures attempts were made to measure 'predation pressure' in treated and untreated plots. House fly pupae and aphid parasitoid mummies were attached to 5 cm×5 cm pieces of emery cloth and placed on the ground in the plots for 24 h periods, at approximately two week intervals from late May to mid-July and the number eaten were counted. Consistently more mummies were eaten in plots treated with organic matter than in untreated plots. More fly pupae were also eaten in treated plots in late May and the first half of June, but this effect disappeared later in the season. Thus, predation pressure was greater in plots treated with organic matter than in untreated plots, particularly early in the summer and particularly for small, aphid-sized prey items. In most years cereal aphid populations, especially of *S. avenae*, reached higher levels and declined more slowly in the untreated plots than they did in treated plots.

The two types of prey used in the field trials were also exposed to a range of beetle predators in Petri dishes in the laboratory to determine which species were prepared to feed on them. Only the larger species (*Pterostichus* spp., *Harpalus rufipes* and *Nebria brevicollis*) ate the fly pupae but all the species tested ate the mummies. (Powell; Ashby and A. F. Wright)

Pests of oilseed and legume crops

Oilseed rape pod midge. The brassica pod midge (*Dasineura brassicae*), an important pest of oilseed rape, causes premature shattering of pods and loss of seed. The phenology of its emergence from overwintering sites and its subsequent infestation of winter oilseed rape crops on a farm in southern England has been studied over the past three years. Two generations occurred each year. The first generation of adults emerged from mid-May or early June until early July from overwintering cocoons in soil at sites where oilseed rape had been grown in the previous year or years. Many females and a few males migrated to flowering winter rape crops where eggs were laid in the pods. Mature larvae dropped daily

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from the pods from early or mid-June until late July or early August, and formed cocoons in the soil. The second generation of adults emerged from late June until mid-July or early August to lay further eggs in the crop. Larvae from these eggs dropped to the soil to diapause within cocoons for up to three years. (Williams and Martin)

Previous olfactometer studies and experiments with traps baited with virgin female brassica pod midge or with crude extracts of females have shown that virgin females produce a male-attracting pheromone (*Rothamsted Report for 1984*, 94). Delta traps baited with live virgin female brassica pod midge, empty delta traps and sticky traps have now been compared for their effectiveness in monitoring brassica pod midge populations at two emergence sites and in a crop of winter rape. All three trap types indicated a similar pattern of adult flight activity. The virgin female traps caught specimens earlier at one site, and in greater numbers at all sites than the non-attractant traps, and because they caught brassica pod midge selectively, their catches were easier to sort. Trap catches were correlated with the overwintering population densities at the emergence sites. 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, Walton and Cook)

Potential pests of lupin and sunflower. Preliminary studies have been made of the insect fauna occurring on sunflower and white lupin. Insects on plots at Rothamsted and Woburn were monitored regularly by visual inspection, sweep-netting, and a range of trapping methods. Trial crops in southern and eastern England were also visited.

On lupins two generations of pea and bean weevil (*Sitona lineatus*) adults caused leaf notching, and typical larval damage to root nodules was found. At part of the Woburn site 10% or more of lupin seedlings were severely damaged by a boring dipterous larva. The lupin aphid (*Macrosiphum albifrons*) was present at all sites from mid-July until the end of August; its distribution was patchy both within and between sites and peak infestation occurred in the first half of August reaching more than 1500 individuals on some plants. Of other aphids on lupin only *Aphis craccivora* was numerous.

On sunflowers colonies of the following aphids occurred: *M. persicae, Macrosiphum euphorbiae, A. fabae, Aphis* sp. and *Brachycaudus helichrysi*. The latter was present from the end of June until shortly before harvest, and infestation was particularly heavy on maturing seed heads. Very large populations of pollen beetles (*Meligethes aeneus*) occurred on the first sunflower head to open (up to 350 per head) but were unlikely to have caused yield loss.

Polyphagous lepidopteran larvae were found on both lupins and sunflowers (tortricids, noctuids and lymantrids) as were mirid bugs, leaf-hoppers, leaf-miners (Diptera and Hymenoptera) and thrips, all causing only minor damage. Predatory chrysopids, coccinellids, anthocorids and syrphids were found on both crops. (Ferguson)

A study of the pollination requirements of lupins has begun. (Williams, Ferguson and Martin)

Pea moth monitoring behaviour

Monitoring the moth in vining peas. Following an extensive trial (*Rothamsted Report for 1982*, Part 1, 97–98) there now exists the basis of a predictive pheromone monitoring system for use in vining peas. Unlike dry-harvested peas, timing of sprays is not a problem; instead farmers need to know whether or not to spray at full flower.

Cumulative catch of moths by full flower can be used to predict the risk of damage if the crop is not sprayed. On the basis of this risk assessment and their previous market experience, farmers should be able to decide the need for sprays more easily and effectively.

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(Garthwaite, Wall with Greenway, Insecticides and Fungicides and Mr A. J. Biddle, Processors' and Growers' Research Organisation)

Orientation behaviour of male moths. One of the basic assumptions of a mathematical model (*Rothamsted Report for 1982*, Part 1, 284) to describe the orientation behaviour of male pea moths responding to a pheromone source in a dense arable crop is that the males fly upwind when stimulated by pheromone. The finding that moths can use pheromone absorbed on to vegetation (or plastic 'grass') to locate the previous position of a pheromone source which has been removed (*Rothamsted Report for 1982*, Part 1, 97) provided the opportunity to test whether stimulated males would still fly upwind even if the chemical gradient of the pheromone 'trail' is not aligned with the wind.

Plastic 'grass' on a 1 m diameter revolving table set at crop height in a wheat field was exposed to pheromone (100 μg (*E*,*E*)-8,10-dodecadien-1-yl acetate) from a source placed on the upwind edge of the table for half to one hour.

After removal of the source, male pea moths arrived at the table and flew upwind across the table to the exact previous position of the source. If the table was revolved so that the chemical gradient on the 'grass' was in the opposite direction to the wind, moths arriving at the table still flew consistently upwind across the table. Some moths which arrived at the previous site of the pheromone source would spend some time there (and often fanned), but nearly all of them subsequently flew upwind and down the chemical gradient across the table.

The behaviour of males flying upwind across the table was similar regardless of the orientation of the chemical 'trail'. This was shown by video recording and detailed analysis of individual tracks. These results support the idea that males fly upwind when stimulated by pheromone, and demonstrate that local fluctuations of pheromone concentration have little effect on this behaviour. (Wall with Perry, Statistics)

Honeybees

***Varroa* and virus dynamics.** Acute paralysis virus (APV) is a primary cause of both adult bee and brood mortality in European bee (*Apis mellifera*) colonies severely infested with the parasitic mite *Varroa jacobsoni*. In Britain, where the mite does not occur, APV has never been associated with mortality of field bees. The factors affecting the initiation of virus replication and its spread and persistence in both honey bee and mite populations are incompletely understood. Virus replication was induced experimentally in adult bees by the injection of foreign proteins and in nature digestive enzymes secreted by the mite may have a similar effect. Alternatively, virus may be released from tissues in which it is normally contained when cells are damaged by the mite feeding. Work on these aspects of the initiation of virus replication will continue. Evidence from both field and laboratory studies indicated that once APV started to multiply systemically in adult bees the mite could then act as a virus vector. Preliminary results suggested that mites are capable of transmitting APV up to two days after feeding on infected hosts and could infect more than one subsequent host. Work in collaboration with European colleagues monitoring APV levels in both live adult bees and mites by enzyme-linked immunosorbent assay (ELISA) (*Rothamsted Report for 1985*, 99) will give further insight into the complex association between *Varroa* and APV. (Ball and Allen)

Honeybee queen recognition. Worker honeybees are able to distinguish by scent a foreign queen from their own and, by attacking the intruder, defend the colony from being usurped. However their behaviour creates difficulties for beekeepers who wish to replace failing queens. Information about the origin of the distinctive odour would be helpful in devising

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more successful methods of introducting new queens to colonies. Odours present in the hive atmosphere become absorbed into the cuticular waxes of all members of a colony and are in part responsible for the colony identity, but there may be an inherited element. To investigate this, queens were caged separately with identical groups of sibling workers. The cages were kept in the same environment and exposed to the same odours for 10 days. Subsequently in choice-chamber tests the workers preferred the queen with which they had been caged, confirming that she had a distinctive odour which was probably inherited. (Free; Ferguson and Simpkins)

Earthworms as indicators of heavy metal bioavailability

Over a two year period, evaluation of the earthworm *Eisenia foetida* as an indicator organism for assessing heavy metal bioavailability has continued. Earthworms were kept in soils and dredged sediments containing elevated concentrations of at least one of the elements Zn, Cu, Cd and Pb. Uptake of these elements into the earthworm tissue was measured at 15, 28 and 56 days. There was a significant and positive correlation between increasing Cu and Cd concentrations in the earthworm tissue and increasing diethylenetriaminepenta-acetic acid (DTPA)-extractable fractions of Cu and Cd in the soils/sediments. Increasing Pb concentrations in the earthworm tissues were significantly and positively correlated with increasing total Pb content of the soils/sediments, but there was no consistently significant relationship between earthworm and soil Zn concentrations.

E. foetida is easily cultured in the laboratory and commonly used for laboratory bioassays. However, it is unlikely that this species would colonize dredged material at disposal sites, so it was necessary to compare uptake of metals by *E. foetida* with uptake by other common field species of earthworm. Although absolute concentrations of metals in the earthworm tissues may differ between species, the results showed a significant positive correlation between metal uptake by *E. foetida* and each of the species *Lumbricus terrestris, Allolobophora longa, A. caliginosa* and *A. chlorotica,* species commonly collected at dredged material disposal sites.

For maximum protection of the environment the species selected for use in a laboratory uptake study should represent the 'worst case' of metal uptake by the group. Results from these studies showed that as the metal concentration in the soil/sediment increased, metal concentration in the tissues of *E. foetida* also increased more than concentrations in the other field species. This confirms that *E. foetida* is the most sensitive species of earthworm for indicating heavy metal availability.

Results of laboratory uptake studies using *E. foetida* are to be compared with metal concentrations measured in native earthworms collected from dredged material disposal sites in the USA. Metal concentrations measured in major groups of soil invertebrates (Coleoptera, Araneida, Isopoda, Diplopoda, Orthoptera) collected at the sites by pitfall trapping will also be used to validate the earthworm test and to provide some indication of levels of heavy metals at different trophic levels of food chains at the sites. (Stafford with McGrath, Cosimini and Fearnhead, Soils and Plant Nutrition)

Staff and visiting workers

During the year J. B. Free retired, the posts of three staff, Patricia Brobyn, M. P. Bentley and R. P. Hadley, were declared redundant, and K. E. Fletcher was transferred to NVRS. Their varied and important contributions to the studies and life of the Department over many years are readily acknowledged.

Thirteen staff participated in International and UK Society meetings and working visits. Visits abroad included: an assessment of locusts programmes in East Africa by W. Powell;

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ARFC/INRA aphid meetings in France by N. Carter, R. Harrington, E. D. M. Macaulay, G. M. Tatchell and I. P. Woiwod; pheromone advisory work at the University of Vicosa, Brazil by C. Wall; *Varroa* studies in the Netherlands and West Germany by Brenda Ball; field sampling for heavy metal pollutants in the Netherlands and USA by Elizabeth Stafford; IOBC modelling meeting in the Netherlands by N. Carter and D. Morgan; IV International Colloquium of Invertebrate Pathology, the Netherlands by M. F. Allen, Brenda Ball and Susan Mardell, and an appraisal visit by T. Lewis to the Beijing Academy of Agricultural and Forestry Sciences, Peoples' Republic of China. Several staff contributed to the British Crop Protection Council Meeting in Brighton and the EEC Meeting on Integrated Crop Protection in Cereals held at GCRI; R. Harrington was co-organizer of a meeting of the European Association for Potato Research, Cambridge.

G. A. Bent, D. G. Garthwaite, E. R. L. Fisher and Susan Mardell resigned, and Caroline Mitchell transferred to RESCU. D. R. Cuthbertson, J. Hargreaves, Gillian Shephard, Janet Southwood and A. J. A. Stewart joined the Department, and nine students or visiting workers joined for varying periods: W. Budenberg, Janette Cook, Julia Hanmer, Pauline Lacey, Ute Seibt, M. P. Walton, Marie Winder, Ho Bun Wong and Xi Long Zhou.

G. A. Bent was promoted to SSO and M. F. Allen to SO.

T. Lewis was awarded a DSc (Nottingham) and became a member of the Research and Development Committee of the Potato Marketing Board.

INSECTICIDES AND FUNGICIDES DEPARTMENT

This year saw the consolidation of the five main Departmental research groups after the reorganizations described last year (*Rothamsted Report for 1985*, 103). Close links with outside agencies have brought in further research funding particularly from the agrochemicals industry and the British Technology Group. Long standing studies on lipophilic insecticides and on insecticide resistance, which have recently provided many new discoveries, are now receiving even greater attention from the agrochemicals industry because of rapidly increasing insecticide resistance in the UK and worldwide. Existing expertise in the physicochemical parameters group has proved invaluable in identifying the underlying causes of damage to crops such as sugar beet by sulphonylurea herbicides following their use on autumn cereals. The commissioning of new glasshouses has coincided with the need for considerably more plant production resulting, for example, from the successful demonstration for the first time that plant-derived antifeedants can be used to control aphid-borne virus disease in the field. This year also saw the commissioning of new nuclear magnetic resonance and mass spectrometry equipment. Both are now working well and are providing essential support to the chemical work of the Department. This equipment was particularly valuable in enabling the first identification of an aphid sex attractant pheromone. Although the existence of such pheromones has been known for many years, identification depended on the new types of electrophysiological recording techniques recently developed in the Department combined with access to the new sophisticated spectroscopy equipment. Unfortunately loss of staff through voluntary premature retirement and redundancy has caused a reduction in studies on the effects of pesticides on beneficial insects and in chemical support for systems for applying novel crop protection agents.

Relationships between molecular structure and insecticidal activity

Fundamental structure-activity studies, based on organic synthesis and bioassay, complement the approach adopted by industry and constitute a powerful technique for identifying structures with potentially useful properties. As opportunities arise, the new compounds are examined for activities against resistant strains of insect, and for selectivity between pests

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and predators. Such approaches may lead to pesticides with more acceptable environmental properties, in particular low application rates and rapid biodegradability.

Synthetic pyrethroids with a non-ester central group. Earlier studies (*Rothamsted Report for 1984*, 102), reporting on the effect of variants used to replace the central ester group in pyrethroids, have now been extended to central units containing ketonic groups.

Synthesis was from an acid derivative (for the $COCH_2$ unit) or from an aldehyde (for the CH_2COCH_2 unit). Bioassays revealed moderate activities for these compounds, but only for particular combinations of central unit and 'acid' component.

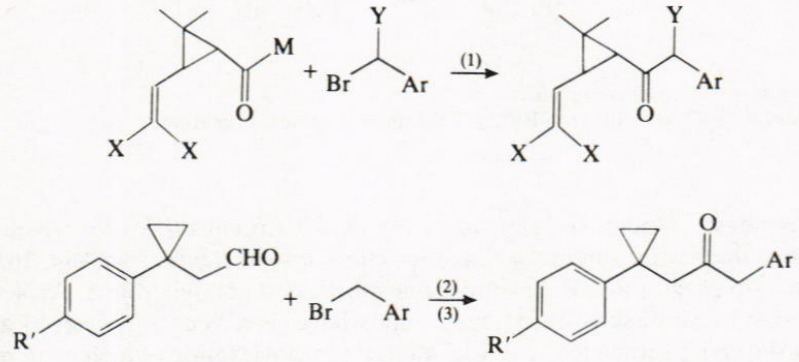

Fig. 1. X=Cl or Br; Y=H or CN; M=Cl or -N(Me)OMe; Ar=3-phenoxyphenyl or 4-fluoro-3-phenoxyphenyl
Reagents: 1) Mg or lithium di-isopropylamide; 2) Mg; 3) CrO_3/H_2SO_4

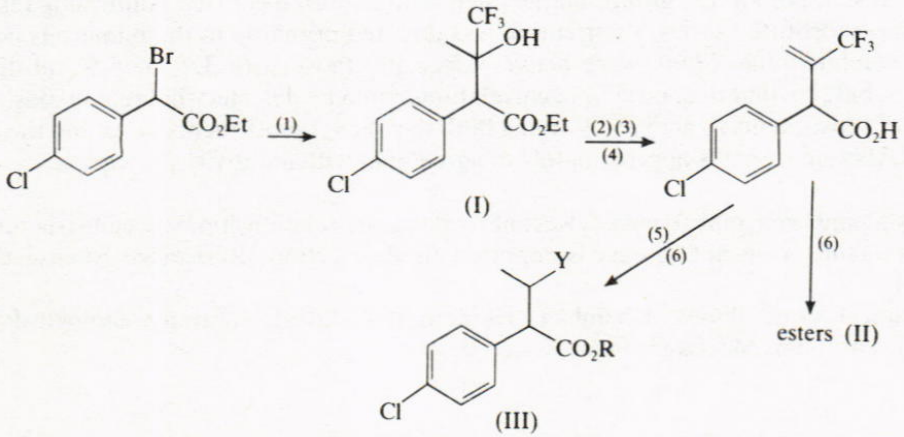

Fig. 2. Reagents: 1) CF_3COCH_3, Zn/CuBr/Et_2AlCl; 2) $POCl_3$/pyridine; 3) OH^-; 4) lithium di-isopropylamide; 5) Pd/H_2; 6) esterification with $ArCH_2OH$ (Ar as above) or their α-cyano analogues.

Trifluoromethyl compounds. The replacement of hydrogen by fluorine in biologically active compounds is a potentially informative technique because, while the size and shape of the molecule are changed little, the electronic distribution is severely perturbed.

The action of compounds such as fenvalerate (III; Y=CH_3, R=α-cyano-3-phenoxybenzyl) is thought to involve the gem-dimethyl group. Analogous compounds with CF_3 groups at Y were prepared by the route shown involving a key alcoholic intermediate (I).. For the saturated compounds (III; Y=CF_3), the two diastereomeric forms were separated. All the products (II and III; Y=CF_3) showed sufficient insecticidal activity to establish that

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the CF_3 group is an acceptable replacement for the CH_3 group at the site of action. Steric properties therefore appear to be more important than electronic distribution in this region of the molecule. Information of this type is helping to build up a picture of how the molecule interacts with the site (as yet uncharacterized), thought to be on, or near to, the sodium channel in the insect nerve.

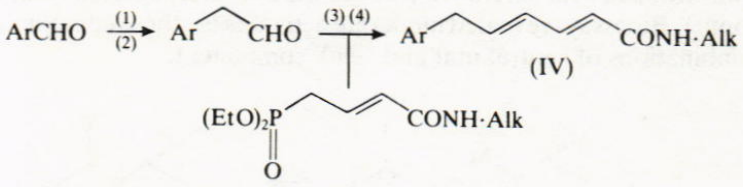

Fig. 3. Ar=mono- or di-substituted phenyl
Reagents: 1) $MeOCH{=}PPh_3$; 2) H^+; 3) BuLi; 4) chromatographic purification

Insecticidal amides. One of the regions of the model structure (IV) for which structure-activity relationships were summarized earlier (*Rothamsted Report for 1984*, 103) has now been examined in greater detail. Several monosubstituted phenyl groups, Ar, led to insecticidal compounds, so di-substituted compounds have now been synthesized and tested. Synthesis by the route shown led to *N*-(2,2-dimethylpropyl)amides which were more easily synthesized without rearrangement of the diene system than were the corresponding *N*-(2-methylpropyl)amides. Preliminary work had established that these two *N*-alkyl groups were both biologically effective in this series.

The presence of methyl groups on the phenyl ring lowered activity, confirming results in the mono-substituted series, so attention was directed primarily to the numerous possible dihalo combinations. Many were active, especially those with 3,4- or 3,5- substitution patterns, but, beyond this, no simple correlations could be detected that related position or nature of substituents to activity. Varying both the phenyl substituents in Ar and the amine group (Alk) gave results approximately in agreement with additivity principles.

Activity against resistant strains. Recent work on the relationship between structure and activity against resistant insects is reported in the section 'Resistance to insecticides' (below).

(Chemical work: Janes, Khambay; Holden, Ifill, Javed, Johnson. Biological work: Sawicki, Farnham; Morrison, Peel, Robertson)

Resistance to insecticides

Research into the problem of containing insecticide resistance continued on two fronts. The direct method is exemplified by the increased sensitivity of *kdr*-pyrethroid-resistant houseflies (*Musca domestica*) to *N*-alkylamides, a potential means by which chemicals can control resistance and by studying the selection of resistance in aphid populations by different insecticides. In the indirect approach, predicting the outcome of different control treatments by laboratory simulations provides means for identifying the operational parameters that determine selection rate. Work is being extended to include the whitefly *Bemisia tabaci*. Improvements in resistance monitoring of field populations by immunoassay have revealed a substantial increase in the frequency of strongly resistant variants of the peach-potato aphid, *Myzus persicae*, which are likely to threaten seriously the effective control of this species in the field.

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Resistance mechanisms in the housefly

***New variants of* kdr *or* super-kdr.** Following the discovery of two variants of *super-kdr* in the housefly, identified by their cross-resistance characteristics to the pyrethroids (*Rothamsted Report for 1985*, 108), additional *kdr* or *super-kdr* variants were sought in housefly strains from the UK, Europe, America and Africa. Where the cross-resistance characteristics revealed by bioassay appeared anomalous, the III autosome, which carries the *kdr* factor, was genetically transferred into the susceptible background for more detailed cross-resistance assays, done in this laboratory, and for neurophysiological studies by Miss A. Gibson, University of Birmingham.

As yet, no correlation between the presence/absence of *kdr* or *super-kdr* and location or previous insecticide usage is apparent. The same holds for *pen*, the penetration delaying factor, which is also located on autosome III.

Nature of resistance to* N-*alkylamides in field strains. An insecticidally active *N*-alkylamide, BTG 502 (IV; Ar=5-bromo-2-naphthyl, Alk=1,2-dimethylypropyl), was bioassayed with and without piperonyl butoxide against adults of twenty insecticide-resistant populations of field-collected houseflies. Piperonyl butoxide strongly synergized BTG 502 even against the susceptible strains, and eliminated the resistance shown by most of the populations tested, except in one strain (designated Ain) which very strongly resisted (>500-fold) the combination. It is assumed that, in most strains, resistance to *N*-alkylamides is caused by mixed function oxidases, which are inhibited by piperonyl butoxide.

***Positive and negative cross-resistance between pyrethroids and* N-*alkylamides in strains with* kdr *or* super-kdr.** Negative cross-resistance between the pyrethroids and *N*-alkylamides (*Rothamsted Report for 1985*, 105) was confirmed in seven of the eight strains with *kdr* or *super-kdr* of different origins. However in the eighth strain, 5866 whose III autosome was derived from strain Ain, there was not only resistance to pyrethroids through *kdr* but also strong resistance to *N*-alkylamides. Thus the non-synergizable resistance to *N*-alkylamides present in strain Ain is on autosome III. The nature of this resistance is being investigated.

Change in resistance characteristics of strain 5640. During 1985–86 an unexplained change in the resistance characteristics of strain 5640 to deltamethrin and WL 48281 (the dichloro analogue of deltamethrin) was observed. Whereas previously the combination of factor 161 and *super-kdr* present in this strain was far less effective against WL 48281 than against deltamethrin (Sawicki *et al.*, *Pesticide Science* (1986) **17**, 483–488), there is no longer any difference in resistance to these two compounds—strain 5640 is now virtually immune to both. Selections with deltamethrin done during 1985–86 may have introduced additional resistance, but the very strong difference in resistance levels between males and females and good synergism by propyl prop-2-ynylphenylphosphonate (NIA) has not changed.

Modelling the dynamics of resistance development. Laboratory work on the response of houseflies with and without the pyrethroid resistance mechanism *kdr* has continued to resolve factors influencing selection for resistance in this species. This has now yielded sufficient data to model aspects of selection for *kdr* by pyrethroid residues, and compare the predicted and observed outcome of control treatments against age-structured fly populations, as described below. These studies demonstrate some of the prerequisites for the realistic modelling of selection for insecticide resistance, and of possible resistance-countering strategies.

Response of houseflies with* kdr *and* super-kdr *to pyrethroid residues. The extent to which application methods modify the phenotypic expression of *kdr*, already evident from compar-

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ing topical and spray-chamber tests (*Rothamsted Report for 1985*, 108), became further apparent with permethrin applied by a standard residue bioassay to different genotypes at the *kdr* locus. The resistance factor between susceptible and homozygous *kdr* strains for permethrin was high (19×) in a topical application test, but lower (6×) in a test where flies were confined over a treated surface. The difference between *super-kdr* and *kdr* in the residue tests was also greatly reduced (10×, compared with 48× in the topical tests). Moreover, since *kdr* heterozygotes showed approximately twofold resistance in both types of bioassay, the degree of dominance of the *kdr* allele, relative to the response of the resistant homozygote, was much greater in residue (0·27) than in topical tests (0·09). These results emphasize the need to ensure that experiments on selection for resistance reproduce closely the means by which insects encounter insecticides in the field.

***Selective advantage conferred by* kdr.** The relationship between residue level and the selective advantage conferred by *kdr* under free-flying condition in population cages was investigated by measuring the survival rates of adults of known *kdr* genotype in cages containing permethrin residues at concentrations up to and including the recommended field application rate. Whereas susceptible flies were readily killed by doses equivalent to one sixteenth of the field rate, *kdr* homozygotes were only substantially affected by concentrations close to the field rate itself. This clearly showed why permethrin, even when correctly applied against populations containing *kdr,* leads to rapid development of resistance.

Computer modelling of selection rates. Existing computer models of insecticide resistance deal primarily with discrete treatments against insect populations with non-overlapping generations, and so are inadequate to describe selection in age-structured populations experiencing continuous exposure to insecticides throughout the life-span of adult flies. Hence to model realistically the outcome of different control treatments, a purpose-built program that uses empirical data on the response of each genotype and sex to predict changes in both *kdr* frequency and population size was developed. Initial trials of the model have reproduced satisfactorily events observed in selection experiments in the laboratory and field.

Biochemistry of resistance

Study of housefly acetylcholinesterase and the carboxylesterase E4 that causes resistance in *Myzus persicae* continued. Since resistant aphids contain large amounts of E4, an immunoassay of the enzyme has been exploited (*Rothamsted Report for 1983*, Part 1, 104) for monitoring resistance both in field crops and in field experiments to understand how resistance develops in aphid populations under insecticide selection. This increased enzyme production is also being studied by molecular biological techniques to understand its genetic basis. Previous studies point strongly to gene amplification as the mechanism responsible, and this year's work has provided more evidence to support this hypothesis.

Further use of an immunoassay to quantify frequencies of resistance in* Myzus persicae *populations. Unenclosed field experiments on sugar beet at Broom's Barn were monitored to determine the effects of aphid immigration on rates of selection after a single application of insecticide. The initial rapid selection for very and extremely resistant aphids (R_2 and R_3 respectively), especially by pyrethroids, was reduced in subsequent samples by an exceptionally large influx of more susceptible (S and R_1) alate aphids. Immigration is therefore important in maintaining the low levels of resistance, but its future impact is dependent on continued high proportions of relatively susceptible aphids in the national population.

It is therefore of particular concern that recent intensive monitoring of sites in southern

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and eastern England in autumn 1985 has shown a large increase in the frequencies of R_2 aphids on a variety of unsprayed crops. R_3 aphids, previously only associated with glasshouses, have also now been found at low frequencies at a number of unsprayed sites. Frequencies of R_2's ranged from 0·10 to 0·49 this year compared with 0·02 to 0·04 during 1980–84 (Mr C. Furk, MAFF, Harpenden Laboratory). Control failures on repeatedly sprayed potatoes in East Anglia, which caused localized areas of defoliation, were also associated with frequencies of highly resistant aphids (R_2 and R_3) approaching 1·00. (with Loxdale, Entomology)

***Genetic basis of insecticide resistance in* M. persicae.** Further screening of a cDNA library (*Rothamsted Report for 1985*, 109) identified 3 clones that hybridized more strongly to the mRNA of resistant than of susceptible aphids. One of these, pMP24, hybridized to an mRNA of the size (2kb) expected to encode E4. Furthermore the extent of its binding to aphid DNA correlated with the insecticide resistance of the aphid strain from which the DNA was extracted. These studies thus provide direct evidence that resistance is associated with gene amplification, although it is not yet unequivocally identified as involving the E4 gene. To gain a better insight into this amplification, we screened a genomic library from very resistant aphid DNA, for hybridization to the insert of pMP24 and isolated several clones that share homology with this plasmid. Hybrid selection techniques have not yet identified the protein corresponding to these cloned sequences and are therefore being complemented by examining the amino acid composition of E4. After radiolabelling its catalytic centre, the protein was cleaved with cyanogen bromide to give six fragments, as expected from its previously established methionine content. One of these, containing the catalytic centre, was purified by SDS-PAGE and partially sequenced. This information will be used to help identify the E4 gene by using oligonucleotide probes complementary to the sequence. (with B. G. Forde, Biochemistry)

Insecticide-insensitive acetylcholinesterase in housefly populations. Of the four mutant types of acetylcholinesterase previously isolated in homozygous form in housefly strains, only one (from strain 2985, derived from a Danish strain in the 1970s) was insensitive (three-fold) to the recently introduced organophosphate, azamethiphos; the remainder were as sensitive as the reference susceptible enzyme or even slightly more (1·5-fold) sensitive. This chemical generally gives good control of housefly populations resistant to other insecticides. However, insects from a population that had survived selection with azamethiphos contained a further form of acetylcholinesterase insensitive to this insecticide, further illustrating the diversity in this important target enzyme for insecticides, and its potential for conferring resistance to newly-introduced compounds.

(Sawicki; Byrne, Dand, Denholm, Devonshire, Dunckley, Farnham, ffrench-Constant, Field, Moores, Peel, Perryman, Rowland, Stribley, Venning, Williams, White)

Compounds influencing behaviour of invertebrates

Aphids remain the most important group of insect pests in the UK, so much of the Department's fundamental work on insect pheromones relates to them. However, with the success of preliminary field trails of the plant-derived antifeedant (-)-polygodial against aphid-borne barley yellow dwarf virus (see the section on 'Systems for application of novel crop protection agents' below), attempts are now being made to extend the types of antifeedant and also the range of insect pests examined. Thus, observations on species affected now include lepidopteran larvae and Coleoptera. In addition, a number of new species of plants, particularly in the family Labiatae, have been investigated for antifeedant activity. The most active examined so far is the ground-pine, *Ajuga chamaepitys*, containing

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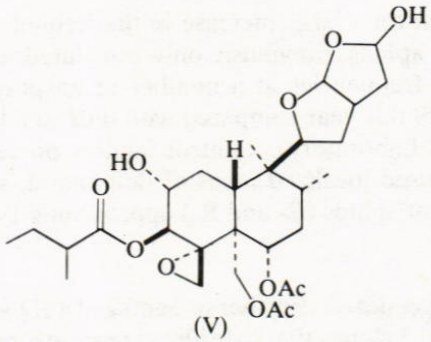

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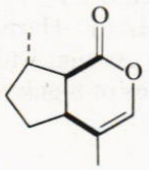

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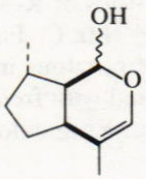

(VII)

the antifeedant 14-hydro-15-hydroxyajugapitin (V). This plant is now being grown on a large scale under glass at Rothamsted. (With Professor F. Camps, Dr A. Guerrero and Mrs G. Fabrias, Institute of Bio-organic Chemistry, Barcelona, Spain)

The sex pheromone of *Megoura viciae*. In extension of work on compounds specifically active against aphids, the first identification of an aphid sex pheromone has been achieved. It has been known for over 15 years that oviparous females of aphids produce a sex attractant pheromone on their hind tibiae but the nature of the active components was not established. Pentane extracts from hind legs of oviparae of the vetch aphid, *Megoura viciae,* were shown to be attractive to males of the species in a laboratory bioassay. Gas-liquid chromatography (GLC) coupled with an electrophysiological recording from an olfactory cell in a secondary rhinarium of the male antenna, showed two electrophysiologically active peaks. GLC coupled with mass spectrometry (MS) gave molecular weights of 168 and 166 for the two compounds (in order of elution from an OV101 capillary column). The later peak was tentatively identified by MS as (4a*S*,7*S*,7a*R*)-nepetalactone (VI) or its enantiomer. This was confirmed by peak enhancement on two GLC capillary columns with an authentic sample of (VI) isolated from the catmint, *Nepeta cataria* (Eisenbraun, E. J. *et al.*, *Journal of Organic Chemistry* (1980), **45,** 3811–3814). The identity of this *N. cataria* material was confirmed by ^{1}H and ^{13}C nuclear magnetic resonance spectroscopy. Reduction of the nepetalactone (VI) with di-isobutylaluminium hydride gave a nepetalactol (VII) which was shown by MS and GLC to be identical with the compound of molecular weight 168 in the aphid extract. Although reduction gave only one isomer of the lactol structure, the absolute stereochemistry at C−1 has not been confirmed. Both compounds, (VI) and (VII), were electrophysiologically active, whereas the enantiomer of (VI), synthesized from (*R*)-pulegone, was inactive. Thus the absolute stereochemistry of (VI) is confirmed as (4a*S*,7*S*,7a*R*). In a behavioural bioassay, which compared aggregation in treated vs. untreated areas, compounds (VI) and (VII) individually were virtually inactive. However, a 1:1 combination of the two components gave a similar response to that obtained with an aphid-derived extract containing the same proportion of the compounds. In both these tests the difference between numbers of aphids on treated and untreated areas was highly significant ($P<0.001$).

This is the first identification of an aphid sex pheromone, and it is interesting to note that the nepetalactone (VI) is already known to be an attractant and excitant for cats and other Felidae; it is also structurally related to compounds acting as attractants for aphid predators in the family Chrysopidae.

Interspecific attraction is reported for the aphid sex pheromone and this is now being investigated for other more economically important species.

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

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Systems for application of novel crop protection agents

The charged, vertically-mounted, rotary atomizer (*Rothamsted Report for 1984,* 111) was used to apply insecticide mixed with alarm pheromone in a volatile solvent against aphids on potatoes. At each of three dose levels, the pheromone-insecticide combination was detectably more effective than insecticide alone. There is still a need to develop an aqueous formulation of the combination that can be applied with conventional spray equipment.

A range of candidate antifeedants was applied by means of charged rotary atomizers to various crops in field tests against several target insect species. In some instances extracts of *Ajuga remota* significantly decreased numbers of aphids on wheat and damage caused by cabbage caterpillars. Polygodial and an aphid alarm pheromone derivative also decreased numbers of wheat aphids to a similar extent. The most effective treatment was polygodial applied electrostatically to autumn-sown barley against aphid transmission of barley yellow dwarf virus. In severely infested plots, virus was decreased from 43% to 25% and yield increased from 3·83 to 5·22 t ha^{-1}.

(Griffiths; Pye, Smart, Woodcock)

Pesticide distribution in plants and soil

Transport of pesticides in phloem is largely dependent on physico-chemical properties. To study the relationships further, work has continued on finding a rapid way of measuring phloem mobility using the model plant *Ricinus communis,* concentrating on techniques that can be adapted for use with non-radioactive compounds.

Undue persistence of chemicals in soil is a matter of concern to both farmers and the general public. This year, investigations have concentrated on the behaviour in soil of two classes of compounds, triazole fungicides and sulphonylurea herbicides, examples of both of which have been reported to be sufficiently persistent to affect following crops.

Phloem translocation. A simple technique has been developed to assess rapidly the efficiency of phloem transport of chemicals using small plants of castor bean *R. communis* (*Rothamsted Report for 1984,* 111). Aqueous solutions of chemical (2-10 mM) were applied by injection into the petioles of the two mature leaves (7 μl in each), and phloem exudate was collected from incisions at the top and bottom of the stem over a period of 2 to 4 h after application. The amount of parent compound in the exudates was assayed either by liquid scintillation counting of ^{14}C-labelled compounds, following separation by thin-layer chromatography, or by high-pressure liquid chromatography (HPLC) of unlabelled compounds. In agreement with previous observations, carboxylic acids and acidic amides having appropriate polarity and pKa were observed to be the best translocated in phloem. The technique using HPLC has been developed to give a rapid indication of the mobility in phloem of a wide range of compounds, such as putative pesticides, at an early stage in a synthesis programme when radio-labelled product would not be available.

Physico-chemical properties of fungicides in relation to effects on take-all. Some fungicides that inhibit ergosterol biosynthesis are among the most toxic to the take-all fungus (*Gaeumannomyces graminis* var. *tritici*) *in vitro,* but even the best of these when tested as soil treatments performed inadequately in the field. Therefore, a range of active fungicides were investigated to discern the influence of physico-chemical properties on activity against the take-all fungus in soil. In agar plate tests the order of toxicity was penconazole > nuarimol ⩾PP969, which is also the order of their lipophilicities as indicated by their octanol-water partition coefficients (K_{ow}). In tests where fungitoxicity was measured after uptake from the vapour phase (to simulate diffusion in the soil), penconazole was ten times more toxic than PP969 from a treated glass surface and twice as toxic from treated soil; nuarimol was not

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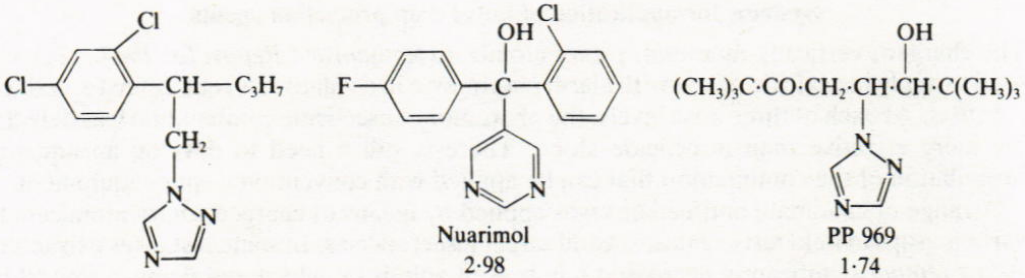

Penconazole
log K_{ow}: 3·20

Nuarimol
2·98

PP 969
1·74

active, being involatile. In pot tests where the chemicals were homogeneously distributed throughout the soil, the effectiveness of the three fungicides differed little. In further tests using alternate layers (depth 2 cm) of treated and untreated soil, the order of toxicity to take-all on the roots of growing wheat plants was PP969 ⩾ nuarimol > penconazole, the reverse of that in agar plate tests and of lipophilicity. In this type of test the greater availability for redistribution in soil water of the most polar compound (PP969), due to its lesser adsorption by soil, therefore outweighed its poorer intrinsic toxicity to the fungus. (with Bateman, Plant Pathology)

A series of analogues of penconazole designed to cover a range of vapour pressures and lipophilicities were synthesized and tested. The same bioassays are being used and several compounds have shown some activity against take-all.

Rates of degradation of triazoles in soil. Despite the widespread use of triazole fungicides, there is little information on the behaviour of triazole compounds in soil or on the effects of residues on fungi associated with following crops. Degradation rates in a clay loam soil were measured for analogues of 1-benzyltriazole and for two commercial fungicides, PP450 and triadimefon. Degradation rates of the substituted benzyltriazoles were in the order H > 4-OCH_3 > 4-F > 4-Cl ⩾ 4-*tert*-C_4H_9 > 3,4-Cl_2 > 3-CF_3, and this could be largely explained by the electronic, steric and polarity effects of the substituents. The effects of temperature and soil water content on rate of degradation were studied for 1-benzyltriazole and 1-(4-fluorobenzyl)triazole. The absence of an Arrhenius relationship and the observation of a lag phase in the degradation of 1-benzyltriazole together provide evidence that breakdown is largely microbial.

Chlorsulfuron damage to sugar beet. ADAS invited the Department to investigate the cause of damage to sugar beet grown in 1986 following application of the sulphonylurea herbicide chlorsulfuron to cereals in the autumn of 1984. Previous investigations (*Rothamsted Report for 1985*, 112) and recent simulations of chlorsulfuron movement through soil for this period showed that, in a free-draining soil of moderately high pH, chlorsulfuron could have leached to depths of more than 1 m. Although some residues might have persisted, dilution caused by this substantial leaching had originally been thought to make it unlikely that following crops could be damaged.

To identify the factors involved in the observed damage, the location of incidents of herbicide damage notified to the British Sugar Corporation were plotted on soil maps. Eighty-eight cases occurred on pelosols and stagnogleys which are impermeable, fifty-five on gleys which are intermittently waterlogged, four on peat soils and the remaining eighteen on apparently free draining sites. Although there are uncertainties involved in diagnosis of herbicidal damage, most of the sites where damage has occurred are poorly drained or low

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lying, and only a few cases were reported from the sugar beet growing areas where subsoils are free draining.

The simulations provide evidence that chlorsulfuron applied in autumn can readily be leached below the plough layer to a depth where both microbial activity and chemical hydrolysis (particularly in alkaline soils) could be low. At sites where drainage is impeded, the tapping of this subsoil layer by a deep-rooted and highly sensitive crop such as sugar beet, and/or the return of this water to the topsoil by capillary action, can lead to appreciable damage. With the recent trend to autumn sowings of cereals, the possibility exists of similar incidents with other acidic herbicides and the appearance of such chemicals in drainage waters following leaching.

(Bromilow; Chamberlain, Evans, Hopkins, Nicholls, Patil)

Staff of the Department

J. H. Stevenson transferred to the post of Station Information Officer. In 1983, he was acting Head and thereafter continued to manage much of the day to day running of the Department whilst maintaining his work on the effects of pesticide use on beneficial insects. G. R. Cayley left as his post was declared redundant; his dynamic approach and broad expertise in analytical chemistry will be much missed. Dawn Wells retired after 10 years' conscientious and valuable service in the Department.

R. J. Lewthwaite and A. R. Gooding left at the termination of their temporary contracts. In other such posts, financed by the British Technology Group, A. J. Robertson, Louise M. Morrison, A. G. Peel, and A. Baydar left, and Jacqueline Holden and A. D. Ifill joined. Pauline A. Dand left and was replaced in the AFRC New Initiative post by Rachel L. Dunckley. Sarah A. M. Perryman left and was replaced by Janet R. Williams. F. J. Byrne and Barbara S. Hackett were appointed to work on insecticide resistance in whitefly, in posts funded for two years by Ciba-Geigy. Work funded by BASF on the behaviour of metazachlor in soils was successfully completed.

We are pleased to report that Linda M. Field was awarded a first class honours degree from the Open University.

We welcomed several scientists from abroad to work in the Department. Gemma Fabrias, from the Instituto de Quîmica Bio-orgánica, Barcelona, spent one month working on pheromones as part of a collaborative programme supported by a British Council Grant, and Zhang Zhong-ning from the People's Republic of China completed two years' studies on novel antifeedants. S. G. Patil, from the University of Agricultural Sciences, Bangalore, spent six months in the Physico-chemical Parameters Group, and O. R. W. Sutherland, from the Entomology Division, Department of Scientific and Industrial Research, New Zealand, is spending a similar period working on the olfactory behaviour of *Sitona lineatus*.

I. Denholm and R. M. Sawicki (Session Organizer) attended the IV Congress of Protection of Human Health and Food in the Tropics, held in Marseilles, and the latter also attended the Open Meeting of the Pyrethroid Evaluation Group at El Centro, California. J. A. Pickett, A. L. Devonshire (Session Organizer), B. P. S. Khambay, R. M. Sawicki and R. H. Bromilow presented posters at the meeting of the International Union of Pure and Applied Chemistry in Ottawa, and J. A. Pickett and B. P. S. Khambay also gave presentations at several laboratories in the USA. L. J. Wadhams spent two weeks at the University of Lund, Sweden, developing new electrophysiological techniques.

J. A Pickett assumed the chairmanship of the Pesticides Group of the Society of Chemical Industry, and presented papers at the meetings 'Applied Biology: Pathways Forward' organized by the Association of Applied Biologists, 'Pesticides: Where Chemistry, Biology and Industry Meet' organized by the Aberdeen Section of the Society of Chemical Industry and 'Biotechnology's Potential for Increasing Crop Production Efficiency' organized jointly by AFRC, ADAS and the Royal Agricultural Society of England. Additionally, he and

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R. M. Sawicki participated in the COPE Workshop at Imperial College on cotton pests. R. H. Bromilow and K. Chamberlain contributed a paper to the workshop on 'Biochemical and Physiological Techniques in Herbicide Research' organized by the Association of Applied Biologists. D. C. Griffiths and B. J. Pye presented a poster at the meeting 'Sprays and Sprayers' organized by Ciba-Geigy. A. L. Devonshire, I. Denholm, A. W. Farnham and R. M. Sawicki (co-organizer) presented papers at the symposium on 'Fundamental and Practical Approaches to Combating Resistance', and N. F. Janes, B. P. S. Khambay and L. J. Wadhams presented papers at a meeting on 'Chirality in Chemical Crop Protection' (at which J. A. Pickett was Chairman); both meetings were organized by the Society of Chemical Industry. Also several members of the Department attended the BCPC conference at Brighton, where I. Denholm, A. W. Farnham and A. L. Devonshire were involved in organizing the programme, as well as participating in, and contributing to, several other meetings.

NEMATOLOGY DEPARTMENT

This year has been overshadowed by the tragic death of the Head of Nematology, Dr. Alan R. Stone. Alan had been Head of the Department since 1979 and, despite an onerous administrative burden, had continued work on the taxonomy of round cyst nematodes on which he was a world authority. His period as Head of Department coincided with severe cuts in government funding and he was tireless in his efforts to attract external funds to ensure continued wide coverage of nematological research at Rothamsted. His forceful advocacy of nematology and his contributions to Station activities have been sorely missed.

Substantial commercial funding has been forthcoming to allow research on biological control agents to expand. A SERC/CASE award with the University of Leeds provides for studies on the effects of host physiology on the development of *Ditylenchus dipsaci*, and a joint project with Luton College of Higher Education and Cambridge University will examine the interaction between potato crop physiology and population dynamics of potato cyst nematodes. Further funds to support short-term projects on hatching physiology have been obtained from the Nuffield Foundation. This report presents a brief survey of current research and collaborative projects in the Department, concentrating on areas which have not been highlighted in recent annual reports.

Control measures

The Department investigates various approaches to nematode control, including integrated control with nematicides, the use of resistant cultivars, crop rotation, and biological methods.

Control of stem nematode. On Rothamsted farm, aldicarb or carbofuran applied to the seed furrows at sowing almost eliminated infestation of spring bean stems by the 'giant race' of stem nematode, *Ditylenchus dipsaci*, and minimized infestation in seed raised from an infested seed stock. Electrostatic or hydraulic sprays of thiabendazole, carbendazim or dimethoate applied to the foliage mid-season did not enhance nematode control. In plots infested with the 'oat-onion' race of stem nematode, onion yield was increased from 0·5 t ha^{-1} in untreated plots to 61·8 t ha^{-1} by aldicarb applied around the seed furrows at sowing. Carbofuran, similarly applied, was almost as effective. Thiabendazole, applied to the foliage to improve nematode control in store, had little effect on onion yields; losses in onions stored for five months were small if the plants had been grown in rows treated with aldicarb or carbofuran at sowing.

The lucerne race of stem nematode is an important limiting factor in lucerne growing.

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Carbofuran applied around the seed furrows at sowing markedly improved establishment and yield of the crop in the first and second years on infested land. Part of the benefit is probably due to control of pea and bean weevils (*Sitona* spp.). Foliar hydraulic or electrostatic sprays of carbendazim, dimethoate, thiodicarb or thiabendazole further increased herbage yields in one experiment but not in another. The 'resistant' cv. Euver, sown in seed furrows treated with carbofuran, yielded as well over four years as the susceptible cv. Europe treated with carbofuran and repeated foliar applications of other pesticides.

Glasshouse experiments have shown that the multiplication of stem nematode populations varies greatly between cultivars of lucerne or red clover indicating the presence of different pathotypes or biotypes in different populations of a so-called 'host-race'. New resistant cultivars should be tested against a range of populations before they are released for general use. Of the lucerne cultivars tested, Vertus was resistant to some lucerne stem nematode populations and susceptible to others, while the supposedly resistant cvs Euver and Lifeuil were as susceptible as the susceptible cv. Europe. Amongst red clover cultivars tested, Norseman, and especially Sabtoron, were very resistant to red clover stem nematodes; Renova, Rittinova and Quin were more or less resistant, being susceptible to one or more of the populations to which they were exposed; Britta, Lucrum and Temara were the least resistant and Redhead, Kühn, Changins and Mt. Calme were fully susceptible.

The most promising approach to control of stem nematode in forage legumes would seem to be the combined use of carbofuran, applied to the seed furrows, a reliable resistant cultivar and, when feasible, a reduced period of cropping. (Whitehead, Nichols and Peters)

Integrated control of potato cyst nematodes. Potato cyst nematodes (*Globodera rostochiensis* and *G. pallida*) are best controlled by a combination of crop rotation, the use of resistant and tolerant potatoes and nematicides.

Over a wide range of densities of *G. rostochiensis*, Maris Piper, Désirée and Pentland Dell were equally tolerant of attack, though of differing yield potential, whereas Pentland Crown was intolerant. Oxamyl in the seedbed prevented yield loss due to *G. rostochiensis* in Pentland Crown and greatly increased yields of Pentland Dell and Désirée – more than by controlling *G. rostochiensis* alone. An adjacent but subsequent experiment showed that tolerance is easily and reliably assessed on plots of lightly and heavily infested soil set up the previous year by growing a resistant or susceptible cultivar of potato in lightly infested soil.

The potato cv. Maris Piper is resistant to *G. rostochiensis* but susceptible to *G. pallida*. *G. pallida* is harder to control than *G. rostochiensis* and is becoming more and more prevalent as a result of the widespread growing of Maris Piper. Some populations of *G. pallida* are poorly controlled by aldicarb or oxamyl and resistance to this species derived from *S. tuberosum* ssp. *andigena* or *S. vernei* is usually only partial and controlled by several genes. In two peaty loam soils, clone ZB 35/29 lessened infestation of the soil by *G. pallida*. In the moderately infested soil, the nematode increased somewhat on Caxton (A25/11), Santé, 12290af20,11233ab22 and Morag (11305a2), while amongst thirteen susceptible cultivars nematode increase varied greatly from fourfold on Romano to 25-fold on Kirsty showing that the choice of susceptible cultivar can also affect nematode population increase. Oxamyl lessened increase of *G. pallida* on some cultivars, not on others. Control of *G. pallida* is further complicated by the fact that resistance to it may vary from field to field, as shown in pots of 35 soils from the major potato growing areas of England. More research is needed to find satisfactory integrated management programmes for soils infested with *G. pallida* alone and mixed with *G. rostochiensis*. (Whitehead, Nichols and Peters)

Biological control of cyst and root knot nematodes. Work has continued on the development of the egg parasitic fungus, *Verticillium chlamydosporium* as a biological control agent

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for cyst and root knot nematodes. Initial studies on six strains of the fungus indicated that there were marked differences between their rates of growth and production of conidia and chlamydospores on corn meal agar (CMA). *V. chlamydosporium* has been isolated from eggs of *Heterodera avenae, H. schachtii, H. cruciferae, H. goettingiana* and *Meloidogyne incognita,* and directly from soil. More than 100 isolates have been collected from a range of nematode infested soils and are maintained in pure cultures on CMA. For long term storage, hyphal fragments and conidia were air dried on silica gel using the method described by D. Smith and A. M. S. Onions (*Transactions of the British Mycological Society* (1980) **81,** 535–540); at 5°C, viability has been retained for at least 18 months.

A simple laboratory screening method has been used to test the isolates for their ability to parasitize eggs of *H. avenae, G. rostochiensis* and *M. incognita* on water agar. There were no significant differences in the overall susceptibility of the three nematode species or in the pathogenicity of isolates collected from eggs or soil. However, there was considerable variation (4–63%) between isolates in the proportion of eggs parasitized and isolates with greatest actitivy against one species of nematode were not necessarily effective against another.

Colonization of the rhizosphere of nematode-infected crops would enable *V. chlamydosporium* to increase the amount of inoculum in soil close to the developing females and increase their chances of infection. Isolates of the fungus varied greatly in their ability to colonize the rhizosphere of barley seedlings. For the fungus to be effective, it must survive until the new generation of females and eggs are produced on the roots about five to eight weeks after planting; it is not known whether *V. chlamydosporium* can continue to colonize and survive for such prolonged periods.

V. chlamydosporium is a facultative parasite that can survive as a saprophyte in soil in the absence of cyst nematodes, but little is known of the factors affecting growth and survival in soil. In tests on agar with six isolates, infection of *H. avenae* eggs was significantly greater ($P<0{\cdot}001$) at 15 to 25°C which was close to the optima for hyphal extension, whereas few eggs were killed at 10°C. It seems unlikely that significant levels of parasitism of encysted eggs occurs in soil over winter. Most females and eggs are killed whilst still attached to the host roots when soil temperatures favour fungal growth and the nematode is most susceptible to attack (*Rothamsted Report for 1983,* 114). Conidial preparations of selected fungal isolates were encapsulated in 1% w/v sodium alginate and 10% w/v kaolin mixture added to 0·1 M calcium gluconate. The granules that formed were air dried in sterile conditions for 24–48 h at room temperature. The fungus survived air dried for at least 40 weeks' storage in the laboratory and for 12 weeks in soil. However, growth from the granule was very limited (<1 mm) in soil and such formulations applied at 1% w/v soil failed to control *M. incognita* on tomatoes and *H. schachtii* on sugar beet in a glasshouse test.

The growth of *V. chlamydosporium* was greatly inhibited by concentrations greater than 10 mg l^{-1} of the fungicide carbendazim incorporated into CMA. However, exposure of conidia to ultra violet light at the LD90 (20 s) has produced mutants that are able to grow at 500 mg l^{-1} of the fungicide. The incorporation of carbendazim into agar could produce a selective medium for the isolation of the mutants from soil and may be used to establish the fungus. Tolerance to the fungicide appears to be genetically stable but it is not clear how other important attributes of the fungus have been affected by exposure to UV light.

Other nematophagous fungi have been studied for their efficiency as biocontrol agents. A strain of the nematode trapping fungus *Arthrobotrys irregularis* that is commercially available as 'Royal 350' (supplied by B. V. Koppert, The Netherlands) was tested for its effectiveness in controlling *M. incognita* on tomatoes in the glasshouse. Applications of the fungus were made on rye grain at the recommended rate of 1·4 g m^{-2} soil area four weeks before planting, and second-stage juveniles (3000 juveniles per plant) were added around the roots 10–25 days later. After ten weeks root galling had not been significantly reduced in soil

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treated with the fungus and the nematode multiplied considerably in all pots. *Paecilomyces lilacinus* (supplied by Dr P. Jatala, CIP, Peru) an egg parasitic fungus, applied on barley grain at a rate of 0·5% w/v soil did not control *M. incognita* or *G. pallida* in a similar experiment on tomatoes. (Kerry, Irving, Kirkwood, Moss and Barba)

Natural suppression of beet cyst nematode populations. Fungi were isolated from infected females of *H. schachtii* and *H. avenae* and from roots of sugar beet, on different occasions in two soils. The three most common fungi in both soils were *V. chlamydosporium, Fusarium oxysporum* and *Cylindrocarpon destructans* but the most active species differed on different sampling occasions; *V. chlamydosporium* was more active in the first generation of the nematode, while the other two species were more active in the second generation. Spreading the contents of individual females across water agar resulted in up to three fungal species being isolated from some females. *Nematophthora gynophila* infected more ($P<0·001$) females of *H. avenae* than *H. schachtii,* but infection rates by other fungi were similar in the two nematodes. Infection by all fungi except *N. gynophila* caused premature tanning of the female cuticle. Similar fungal species and numbers of fungi per female were recovered from females of four different ages.

To obtain a measure of the suppressiveness of a soil to nematode multiplication, observation chambers were used to monitor the changes in numbers of *H. schachtii* females developing on the roots and to isolate fungi from infected females. Estimates of suppressiveness were based on the numbers of females killed during the growing season. These ranged from 81% in soil from a sugar beet monoculture where the nematode was known to be in decline, to 36% in a soil where nematode multiplication was known to be high. *C. destructans* was the fungus most commonly isolated from infected females.

Some isolates of *C. destructans* are weakly plant pathogenic and it is not clear from these results whether the fungus actively parasitizes the nematode, or infects the syncytial cell in the root on which the nematode feeds, hence entering the dead female saprophytically. When *C. destructans* and *F. oxysporum* were added to females and eggs of the nematode *in vitro,* both fungi infected large proportions of females but few eggs were infected. Immature females without eggs were completely colonized by *C. destructans,* whereas mature females full of eggs supported little fungal growth as the eggs did not become infected. Both fungi, when added to soil in pots, caused significant reductions (*c.* 40%) in numbers of females developing on sugar beet roots, without affecting plant weights. Cultures of *C. destructans* were placed on roots in a split root container which enabled part of the root system, with developing nematodes, to grow across a Petri dish, covered only by moist filter paper. Only females in direct contact with the fungus became infected, suggesting that this particular strain of the fungus did not colonize the roots but infected the females directly. In an *in vitro* experiment, blocking the natural openings of the female had no significant effect on the numbers infected by *C. destructans* demonstrating that the fungus is capable of penetrating the female cuticle. (Crump)

Nematicide trials with oilseed rape—methods of application. On land infested with the brassica cyst nematode, *H. cruciferae,* substantial yield responses of oilseed rape, cv. Jet Neuf, were achieved by broadcast treatment with nematicides (see below and *Rothamsted Report for 1983,* 115). The costs of protection with nematicides would be reduced if similar yield responses were to result from limited nematicide application within the row to give protection to the crop during establishment. A site was chosen at Wrangle, Lincolnshire, with an infestation of *H. cruciferae* averaging 54 eggs g^{-1} soil. Broadcast treatments of oxamyl at 10 kg a.i. ha^{-1} were applied at drilling time or during the following April. In addition, oxamyl was applied in the row at drilling time to give overall rates of 2·5, 5 and 10 kg a.i. ha^{-1} when every row was treated, and 1·25, 2·5 and 5 kg a.i. ha^{-1} when alternate

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rows were treated. Both the broadcast treatments increased yield to 4·2 t ha^{-1} compared with 3·5 t ha^{-1} in untreated control plots. The row treatments increased yields significantly only at the highest rates, to 4·6 t ha^{-1} at 10 kg ha^{-1} every row treated, and to 4·4 t ha^{-1} at 5 kg ha^{-1} alternate rows treated. These two treatments represent identical rates of application m^{-1} of row. Thus, placement of the nematicide in this way is more efficient than broadcasting and, providing an effective concentration in the soil is established, treating alternate rows improves yield almost as much as treating every row. (Evans and Russell)

Nematicide trials with oilseed rape—responses of different cultivars. Tolerance of nematode attack, whereby certain crop cultivars yield well despite heavy infection, is widely recognized. The oilseed rape cultivar Bienvenu is thought to be more tolerant of *H. cruciferae* than the cv. Jet Neuf (*Rothamsted Report for 1985*, 119) and this was tested in a field trial near Dover in collaboration with ADAS (Wye). A site was chosen in a field which had grown oilseed rape the previous year, and on which the population density of *H. cruciferae* averaged 97 eggs g^{-1} soil. Cvs Bienvenu, Jet Neuf and Mikado were grown in plots 2 m wide by 30 m long which received either no nematicide or aldicarb applied at 4 kg a.i. ha^{-1} immediately before drilling. Yields obtained from the control plots were 2·79 t ha^{-1} for Bienvenu, 1·94 t ha^{-1} for Jet Neuf and 2·39 t ha^{-1} for Mikado. Responses to nematicide treatment were marked, with yields of 3·46, 2·53 and 3·10 t ha^{-1} for Bienvenu, Jet Neuf and Mikado respectively. Bienvenu outyielded the other two cvs and was also the most tolerant of nematode attack. However, final population densities of the nematode averaged 202 eggs g^{-1} soil in control plots and 217 eggs g^{-1} in plots treated with aldicarb, so nematicide treatment provided no control of nematode multiplication. (Evans, Russell with Mr P. Harris, ADAS)

Cyst nematode biology

Departmental research on nematode physiology and hatching mechanisms was highlighted in last year's *Report*; some other aspects are mentioned here.

Interactions between potato cyst nematodes and *Verticillium* spp. The wilt fungus *V. dahliae* is known to interact with potato cyst nematodes (*G. rostochiensis, G. pallida*) to produce 'early-dying' symptoms in plants infected with both organisms, but not all cultivars are affected similarly (*Rothamsted Report for 1982*, Part, 1, 161; *for 1985*, 120). A second species of wilt fungus, *V. albo-atrum*, is usually commoner than *V. dahliae* in cooler soils, but little is known of the distribution of *Verticillium* spp. in the UK and the extent to which 'early-dying' affects crop yields. The reactions of twelve potato cultivars to infection by these two species of wilt fungus, either alone or in conjunction with *G. rostochiensis* or *G. pallida*, were tested in pots. The early cvs Maris Anchor and Maris Peer were the most susceptible, with the rest of the earlies tested (Pentland Javelin, Estima and Wilja) proving as resistant as some of the maincrop cultivars. Désirée was the most susceptible maincrop cultivar tested and Pentland Crown the most resistant. Death almost invariably occurred sooner when plants were inoculated with *V. albo-atrum* rather than *V. dahliae*. (Evans with Dr G. Storey, Luton College of Higher Education)

Root growth of potato cultivars infected with *G. rostochiensis*. Heavy attack by potato cyst nematodes causes stunting of root systems of potato plants but all cultivars are not affected to the same degree (*Rothamsted Report for 1980*, Part 1, 155) and there is little information on changes in the distribution and configuration of potato root systems after attack. Our understanding of how changes in the root system are related to tolerance of nematode attack and interactions with other disease-causing organisms, such as wilt fungi, require more

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detailed information of the effect of nematodes on root system development. Three potato cultivars (Maris Anchor, Maris Peer and Pentland Javelin) were chosen because they are known to differ greatly in susceptibility to wilt fungus and in tolerance of nematode attack. Maris Anchor is the least tolerant of nematode attack and the most susceptible to fungus attack with Pentland Javelin the most tolerant and least susceptible; both have the H_1 gene for resistance to *G. rostochiensis* pathotype Ro1. Soil which was heavily infested with *G. rostochiensis* (150 eggs g^{-1}) was collected from Woburn, Bedfordshire, and half was steam sterilized. Plants of the three cultivars were grown from single sprouts in the two types of soil. Their root systems were carefully washed free of soil, two, four or eight weeks after planting and the numbers and lengths of main and lateral roots were counted and measured. All three cultivars produced just over 30 main roots per plant and this number was not affected by nematodes. However, main roots in nematode infested soil were markedly shorter than those in sterilized soil, particularly after two or four weeks. Effects on lateral root development were more complex: all three cvs produced abundant (between 4.0 to 5·4 cm^{-1} of main root) long and healthy laterals in sterile soil but in non-sterile soil they were fewer in Maris Anchor (1·1 cm^{-1} of main root at two weeks) and shorter in all three cultivars. In Pentland Javelin they were simply shorter, in Maris Peer they were shorter with necrotic tips and in Maris Anchor they were very short and completely necrotic. At later harvests, rather more laterals per cm of main root were recorded in nematode attacked plants, but these were very necrotic in Maris Peer and especially so in Maris Anchor. This breakdown of roots occurred despite the H_1 resistance gene in Maris Anchor and in contrast to Pentland Javelin, which also has the H_1 gene and in which roots always looked healthy. Four weeks after planting, healthy plants of Pentland Javelin, Maris Peer and Maris Anchor had root systems which weighed 36, 49 and 44 g respectively, whilst those plants attacked by nematodes had root systems weighing 11, 5 and 5 g respectively. (Evans and Russell with Dr G. Storey, Luton College of Higher Education)

Tolerance trials with potatoes and potato cyst nematodes. A field trial design in which plots consist of single plants, for assessing tolerance of potatoes to cyst nematode attack using a minimum of planting material, has been described previously (*Rothamsted Report for 1985,* 119–20). A similar design was used to assess tolerance in 12 cultivars and clones in 1986, except that the trial area was divided into four and aldicarb applied at 0,1, 2 or 4 kg a.i. ha^{-1}. Ten tubers of each cultivar were planted in each area on 13 May using a randomized block design and standard plant and row spacings. Tubers were harvested from individual plants at the end of September. The clones and cultivars were ranked for tolerance of nematode attack: firstly, on the basis of the mean yield of the ten plants growing in untreated soil (average population density 154 eggs g^{-1} of *G. rostochiensis*) and, secondly, on the basis of the regression coefficient for a linear regression analysis of cultivar yield on mean yield of all cultivars in the four blocks of the trial. Cara was the most tolerant cultivar whichever methods of assessment was used and clone 11234 4 was the least tolerant, yielding 0·15 kg tubers per plant compared to 2·48 for Cara in the control plots. Clone 13578 2 was the highest yielding breeding line in the control plots, producing 1·15 kg per plant compared with 1·30 and 0·63 for Fiona and Pentland Crown respectively. (Evans and Russell)

Selection of potato cyst nematodes on resistant *Solanum tuberosum* ssp. *andigena* CPC 2802 hybrids. Five populations of *G. pallida* were reproduced for four generations on four cultivars of potatoes bred from *S. tuberosum* ssp *andigena* CPC2802 and considered to be polygenic for resistance to *G. pallida.* The nematode populations were the same as those used by Turner, Stone and Perry (*Euphytica* (1983) **32,** 911–917) and in their experiments showed increased virulence when selected on *S. vernei* hybrids. In our tests the potato cv. A27/20 (Cromwell) showed little resistance to the populations used. Reproduction of the

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nematodes, although varying from year to year, was always more than 55% of that on the susceptible control and was usually more than 70%; no trend in change of virulence was apparent. Reproduction on the cv. A25/11 when compared with that on the susceptible was similar (about 30%) in all four years. The resistance in this cultivar may therefore be an example of horizontal resistance, although further reproduction on the cultivar would be needed to confirm this. All the populations became increasingly virulent on the other two cultivars. The number of cysts on A25/14 as a percentage of those on the susceptible, increased from 20 to 60 and on ZA 59/25 from ten to 30. This was a similar rate of increase to that found by Turner, Stone and Perry on the *S. vernei* hybrids. Because crushed cysts were used as the inoculum, cysts from previous generations were not present and selection pressure was therefore greater than would occur in the field. (Parrott and Payne)

Multiplication of *H. cruciferae* and *H. schachtii* on oilseed rape. The multiplication of *H. cruciferae* and *H. schachtii* were compared over the range 5–20°C on roots of oilseed rape cv. Jet Neuf. Juveniles of neither species were able to invade the roots at 5°C. However, juveniles which were allowed a period of 48 h at 15°C to invade and then transferred to 5°C developed slowly. At 10°C *H. cruciferae* developed faster than *H. schachtii* but they developed at similar rates at 15°C, with females produced within 30 days of inoculation. At 20°C *H. schachtii* developed faster than *H. cruciferae*: juvenile invasion occurred earlier and in larger numbers, less time was taken to complete a generation (from the invasion of roots to hatching of F_1 juveniles). At 20°C, the multiplication rate of *H. schachtii* was 4·6 times that of *H. cruciferae*. Between 30 and 35% of the total number of eggs produced were found in egg sacs in both species. Juveniles required less stimulation and hatched more quickly from eggs in egg sacs than those in cysts. In pot tests, F_1 juveniles invaded the roots of oilseed rape both earlier and in larger numbers from egg sacs than from cysts. However, egg sacs were short-lived and will not contribute to invasion after ten weeks in soil in the absence of a host.

Screening cultivars and breeding lines of rape revealed little or no resistance to either *H. cruciferae* or *H. schachtii* but differences in hatching and multiplication were found between cultivars and lines. Mikado was a good host of both species whilst the early maturing cvs, Midas and Westar, were poor hosts. The differences between hosts were related to the ability of plants to stimulate hatching and of juveniles to invade the roots, with *H. schachtii* achieving greater multiplication than *H. cruciferae* on all cultivars and lines. Bienvenu was found to be more tolerant of both species than other cultivars and lines, whereas Jet Neuf was less tolerant. A pathogenicity test showed that *H. cruciferae* and *H. schachtii* reduced the growth of cv. Jet Neuf at all initial nematode population densities tested (2·5–300 eggs g^{-1} soil). Infected plants had less extensive root systems and smaller shoots and leaf areas than uninfected plants. The calcium content of shoots was greater in infected plants and was related to the extent of damage to the roots by nematodes. At initial nematode densities of 2·5–25 eggs g^{-1} soil *H. schachtii* was more damaging to rape than *H. cruciferae*. Maximum multiplication rates occurred at an initial density of 2·5 eggs g^{-1} soil because greater initial densities damaged roots and restricted the amount of nematode multiplication that could occur. Equilibrium densities were in the range 40–60 eggs g^{-1} soil. (Evans with Mr S. Bowen and Dr G. Storey, Luton College of Higher Education)

Effects of charcoal on root invasion and cyst production of *G. rostochiensis*. Activated charcoal strongly absorbs the active hatching factors in potato root diffusate. Pot tests were done to determine whether the addition of activated charcoal to sterile loam before planting potatoes affected hatch, invasion and multiplication of *G. rostochiensis*. Chitted potato tubers were planted in 10 cm pots containing a known cyst inoculum and 100, 200, 400 and 800 mg activated charcoal per kg loam. Four weeks after planting, there were significantly more unhatched eggs remaining in cysts in pots containing charcoal compared to control pots

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with no added charcoal; there was also a significant reduction of juvenile invasion. However, after 16 weeks' growth, there was no significant difference between control pots and treatments for number of cysts formed or for unhatched eggs; there was also no effect on plant growth.

In experiments using 10 g activated charcoal either as a 'seedbed' (surrounding the tuber) or incorporated into the loam in 20 cm pots, the incorporated treatment gave greater tuber weight and better inhibition of hatch compared with 'seedbed' treatment and controls with no charcoal. Numbers of new cysts produced were similar in all treatments but cysts were smaller from pots with charcoal and contained fewer eggs.

Thus, charcoal reduces the rate of hatch, probably by absorbing the initial root diffusate production, and reduces initial invasion, perhaps because orientation of the juveniles to the source of diffusate is impaired. In the long term, charcoal does not affect the total hatch or the number of juveniles invading but new cysts are smaller. The delay in hatch and invasion may be sufficient for plants to grow large enough to tolerate invasion. Juveniles may also be depleting lipid reserves during this period leading to a decrease in infectivity (*Rothamsted Report for 1985*, 116). (Perry and Beane)

Effects of herbicides on cyst nematode hatch. A substance which alters the permeability of the lipid layer of eggshells may initiate the hatching sequence in cyst nematodes. Kraus and Sikora (*Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* (1983) **90,** 132–139) found that di-allate, the active ingredient (a.i.) of the herbicide 'Avadex', significantly increased the hatch of *H. schachtii*. Di-allate is a thiocarbamate, a group known to cause membrane disintegration and altered permeability in plant cells. The current a.i. of 'Avadex' is tri-allate and we tested the effects of this and the formulated herbicide on the *in vitro* hatch from cysts of *G. rostochiensis* and *H. schachtii*. Neither the formulated compound nor the a.i. at the rates used increased hatch of *G. rostochiensis* or *H. schachtii*. However, the hatch from CFTM56G. rostochiensis cysts in potato root diffusate (PRD) with 4 mg l^{-1} tri-allate was reduced by over 50% and subsequent hatch in PRD alone was also impaired. The action of tri-allate appears to differ from that reported for di-allate.

Using a range of herbicides (cycloate, pebulate, vernolate, chloridazon, metribuzin, lenacil and tri-allate) at recommended field rates and 10^{-2} and 10^{-3} dilutions made up in distilled water did not increase hatch of the two species compared with controls in soil leachate. However, when the treatment solutions were replaced with host root diffusates subsequent hatch was significantly reduced from cysts previously exposed to field rates of cycloate, pebulate, vernolate and tri-allate.

Hatch trials using separate solutions of each herbicide at field rates made up in PRD for tests with *G. rostochiensis* and beet root diffusate for *H. schachtii* gave almost complete inhibition of hatch of both species. After four weeks, the solution were replaced with fresh diffusates containing no herbicides. Cysts of both species previously exposed to cycloate, pebulate, vernolate and tri-allate still gave no hatch whereas hatch inhibition by chloridazon, metribuzin and lenacil pre-treatments was reversible with hatches in diffusates comparable to controls not previously exposed to herbicides. (Perry and Beane)

Taxonomy

Studies on nematode taxonomy are an important part of the Department's work providing essential information for advisory and control programmes and support for research on aspects of nematode biology and pathogenicity. Biochemical approaches support and extend the more traditional morphological studies.

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Morphological studies. An extensive literature back-up, essential for taxonomic work, is maintained and this includes a card index file on the current status of each species of plant and soil nematodes. (Cowland and Hooper)

A very large microscope-slide collection of nematodes is also maintained and it includes some 2000 type slides, many of them deposited from overseas. There is a regular exchange of specimens with researchers in other institutes. The identification of migratory plant parasitic nematodes continues with especial emphasis on longidorid, trichodorid and aphelenchid species. Studies on *Aphelenchoides* species have been supplemented with observations with the scanning electron microscope which has been particularly useful in enhancing features of diagnostic importance with regard to the lateral fields and the shape of spicules. (Hooper and Hoole)

Recent concern regarding the possible introduction of the pine wilt nematode, *Bursaphelenchus xylophilus*, in wood chips from N. America has prompted a check of local pine trees for this nematode which to date has not been recorded in Britian. Various *Aphelenchoides* and *Ditylenchus* spp. were found in timber from diseased and apparently healthy pines but *B. xylophilus* was not encountered. (Hooper)

Biochemical identification

Root-knot nematodes. Root-knot nematodes (*Meloidogyne* spp.) are major agricultural pests worldwide, causing estimated overall average losses to tropical crops of 11–25% and individual total crop losses are not uncommon. There are 49 currently described species of which 13 are of major agricultural importance; the current project aims to develop and modify rapid methods of identification using biochemical techniques.

Several improvements have been made to the sensitivity and practicality of using isoelectric focusing (IEF) to distinguish species of *Meloidogyne* on a routine basis. Ultrathin polyacrylamide gels, 0·15 – 0·3 mm thick, are being used in conjunction with narrow pH gradients for separation of the major species, namely, *M. incognita, M. arenaria, M. javanica* and *M. hapla*. These techniques have improved the sensitivity and clarity of protein profiles from eggs and juveniles and have reduced the cost per gel to less than half that of conventional 2 mm thick gels. Characterization of isozymic differences has also improved the separation of the major species. High sensitivity gold and silver staining is being used to distinguish groups of species which are known to occur together but are difficult to separate morphologically; in particular, those species parasitizing rice in India and Bangladesh and coffee in Tanzania and South America. The Department now holds 20 species and over 40 populations of *Meloidogyne* and a major objective is to produce an atlas of electrophoretograms of soluble proteins of these species for routine identification in conjunction with a handbook on identification by morphometric techniques. (Robinson, with Dr S. B. Jepson, University of Southampton)

Potato cyst nematodes. IEF techniques continue to provide valuable information on the European distribution of cyst nematodes. An analysis of some Portugese populations of potato cyst nematodes using IEF of total soluble proteins revealed the presence of *G. pallida*. This is the first record of this species in Portugal and is an important finding in relation to the country's agricultural practices. (Burrows with Professor Maria de Almeida Santos and Miss Fatima Fernandes, University of Coimbra, Portugal)

Total DNA extraction from potato cyst nematode juveniles. Recent advances in DNA technology enable rapid and reliable analysis of the nematode genome using restriction endonucleases (Curran, Baillie and Webster, *Parasitology* (1985) **90,** 137–144) to give a

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unique 'finger print' independent of a phenotype. The technique required modification for use with potato cyst nematodes as residual egg debris reduces the efficiency of DNA collection and, more importantly, inhibits the endonuclease. Therefore, second stage juveniles were mechanically hatched in a homogenizer with a loosely fitting plunger and the juveniles separated from the empty egg shells by centrifugation of the suspension in a 50% w/v sucrose solution. Eco R1 restriction endonuclease digests of *G. rostochiensis* and *G. pallida* were run on a 0·8% agarose horizontal mini-gel at 100 V for 1 h before being stained with ethidium bromide and viewed under transmitted ultra violet light. Differences in band number and mobility (size) were observed between the *G. rostochiensis* and *G. pallida* populations used. Thus, restriction fragment length differences may differentiate between these species and this approach may provide a powerful new taxonomic tool. (Burrows with Dr S. A. Boffey, Hatfield Polytechnic)

General aspects

Culturing nematodes. The fruit of courgette marrows (*Cucurbita pepo ovifera*) have been used for the mass culture of the oat race and giant race of stem nematode (*D. dipsaci*) originally from field beans (*Vicia faba*). The *D. dipsaci* are injected, using a hypodermic needle, at two to four sites along a moderate sized courgette (20 cm long, 5 cm diameter) which is stored at 15–18°C for two months. The *D. dipsaci* reproduce locally within the courgette tissue which darkens slightly in colour and has a mealy-like texture when heavily infested. Some 20000 *D. dipsaci* have been recovered from 1 g of infested tissue. *Aphelenchoides ritzemabosi* has also reproduced very well in courgettes, migrating throughout much of the tissue; *A. fragariae* reproduced to a lesser extent. (Hooper and Cowland)

Permeability characteristics of *D. dipsaci*. Work is continuing to define the permeability characteristics of the cuticle of fourth stage juveniles of *D. dipsaci*. Experiments were done to examine the rehydration period after desiccation which is required for the cuticle to re-establish normal permeability characteristics. Active, previously undesiccated individuals were exposed to a standard desiccation regime before being rehydrated in distilled water for various periods. All juveniles were then desiccated for 5 min at 0% relative humidity and their individual water content was determined. Juveniles which had been rehydrated for periods between 0·5 and 1·5 h showed little ability to control subsequent water loss. By contrast, juveniles rehydrated for periods >2·5 h were able to control water loss more effectively and thus survive drying. The restoration of normal permeability and survival attributes occurs between 1·5 and 2·5 h rehydration when the juvenile has become fully hydrated after desiccation. Fourth stage juveniles of *D. dipsaci* in bulbs and field beans often survive adverse conditions as dry aggregations called 'eelworm wool'. This work indicates that rapid cycles of wetting and drying bulbs or seeds may adversely affect the viability of juveniles in 'eelworm wool'. (Perry with Dr D. A. Wharton, University of Otago, New Zealand)

Effect of metabolic inhibitors on *G. rostochiensis*. There is very little information on the biochemistry of plant parasitic nematodes, particularly on their pathways of energy metabolism. By investigating the effects of specific inhibitors on metabolism it should be possible to pinpoint pathways which are especially sensitive to perturbation and are, therefore, potential targets for novel nematicides. Three indicators of metabolic activity, heat output, oxygen uptake and ATP levels, have been used to study the effects of metabolic inhibitors on second stage juveniles of *G. rostochiensis*. The results so far indicate that heat output is a more sensitive measure of metabolism than oxygen uptake or ATP levels and has the

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advantage that it is non-destructive and could be automated. By comparing the effects of different inhibitors it can be concluded that there is a major aerobic component in the metabolism of *G. rostochiensis*. Despite this, these nematodes also possess fairly effective anaerobic pathways. (Perry with Professor J. Barrett and Dr P. Butterworth, University of Wales, Aberystwyth)

Staff and visiting workers

The posts of two staff, Janet Fraser and David Tite, were declared redundant; they had been members of the Department for 21 years. Sadly, David died in August 1986 after a short illness. Both had contributed extensively to the field research programme of the Department and David made a major contribution to the development of the Vertical Band granule applicator. M. P. Robinson was awarded a Ph.D. (Leeds University) and is currently employed in the Department on an ODA grant studying biochemical taxonomy of *Meloidogyne* spp. K. G. Davies and Corinna Flynn joined the Department on short term posts obtained from a new research contract.

Shortly before his death, A. R. Stone visited various research centres in the USA. R. N. Perry was invited to the University of Bonn and gave an invited lecture at the Institut für Nematologie, Münster; he also visited the University of Kiel to join in collaborative research. B. R. Kerry attended the cyst nematode workshop and the Society of Nematologists meeting in Orlando, Florida and visited the Agricultural Research Center, Beltsville and the Abbott Laboratories, Illinois; he also gave an invited lecture at the Freie Universität, Berlin. B. R. Kerry and D. H. Crump participated in the IOBC meeting on the integrated control of soil pests in Bonn. R. N. Perry, A. G. Whitehead, D. J. Hooper and M. P. Robinson attended the International Symposium of the European Society of Nematologists in Antibes; D. J. Hooper attended the European Plant Parasitic Nematode Survey workshop in Antibes; M. P. Robinson visited Nematology centres at The Agricultural University, Wageningen and INRA, Antibes; K. Evans attended the European and Mediterranean Plant Protection Organization committee meeting on pathotypes of potato cyst nematodes in Rennes. Staff also attended and contributed to a number of scientific meetings in the UK. K. Evans completed his three year appointment to the Nematology Group Committee of the Association of Applied Biologists and continues to serve on the Association's Editorial Board. D. J. Hooper continues as co-editor of *Systematic Parasitology*.

During the year the Department received many short term visitors, some for training in nematological techniques. Long term Visiting Workers in the Department included Mr D. Jovičić (Yugoslavia: taxonomy), Dr J. Schlang (FRG: spatial distribution of fungal parasites of the beet cyst nematode), Dr G. Storey (Luton College of Higher Education: interactions between potato cyst nematode and *V. dahliae*), Professor Maria de Almeida Santos, Miss Fatima Fernandes and Mrs Isabel Abrantes (Portugal: electrophoresis and scanning electron microscopy for the identification of *Globodera* and *Meloidogyne* spp.), Dr Nalini Gnanapragasam (Sri Lanka: general nematology), Dr U. Zunke (FRG: behaviour of *Pratylenchus*), Dr G. Gurr (NIAB, Cambridge: nematological techniques) and F. de Leij (The Netherlands: cyst nematode/fungal interactions).

PLANT PATHOLOGY DEPARTMENT

The largest single research area in the Department's programme is the study of cereal diseases. This is appropriate, not only because cereals occupy, and will continue to occupy, by far the largest area of arable land in Britain, but also because, as profit margins are squeezed, there is an increasing need to know the consequences of husbandry practices on

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disease incidence and to provide the farmer with the ability to make decisions, based on sound scientific evidence, to ensure an economically and efficiently produced crop of appropriate quality with the minimum of adverse environmental effects. The Department's work is also providing this information on non cereal arable crops especially oilseed rape and potatoes, where programmes investigate a range of fungal and viral pathogens. Other UK crops studied include sunflowers, lupins and peas, and our overseas interests are demonstrated by work on cereal viruses and cloves. Again much of our work is also reported under Multidisciplinary Agronomy.

Diseases of break crops

Oilseed rape

Viruses The incidence of beet western yellows virus infection in plots at Rothamsted was similar (mean 25% both before and after flowering) to that in 1985 but amounts differed less between treatments. Sowing date (16 August or 6 September) had no effect and an autumn insecticidal spray only halved the amount of infection. No infection was detected in the autumn and there was no increase in infection between the pre- and post-flowering sampling dates so, as in 1985, spread must have occurred during late autumn from a few plants infected earlier by migrating aphids. That this spread was less well controlled by insecticide than in 1985 may have been due to the timing of the application in relation to seedling emergence and aphid activity.

Neither cauliflower mosaic virus nor turnip mosaic virus was detected in any sample (Nagarajan; Govier and Cockbain)

Sunflower

Diseases and maturity. Sixty-four varieties and breeder's selections were examined in field plots for earliness to maturity and disease. Despite late sowing (2 to 8 May) 18 reached physiological maturity before the end of September when these earlier maturing types had greater incidence and severity of *Botrytis cinerea* infection on heads (*c.* 85% heads infected) than those maturing later (*c.* 50% heads infected). Infection on the back of the heads first became noticeable in early September and progressed rapidly during maturation; *Botrytis* also caused lesions on stems. Isolated plants were infected with *Sclerotinia sclerotiorum,* which caused either stem or head rot or wilt; *Fusarium avenaceum* caused a head rot and *Alternaria* spp. dark lesions on stems.

Benomyl, chlorothalonil, iprodione, prochloraz, propiconazole and carbendazim+ vinclozolin were each applied twice, at early flowering and at full bloom, using the APE 80 electrostatic rotary atomizer. Only carbendazim+vinclozolin decreased incidence and severity of *Botrytis* infection.

In another experiment six varieties differing in earliness of flowering and maturity were sprayed twice with prochloraz+iprodione+chlorothalonil using the electrostatic atomizer. Sprays were applied at early and late flowering stages, at appropriate times for each variety. These sprays halved the incidence and decreased the severity of *Botrytis* head infection on some varieties but had no effect on others. (Rawlinson; Church with Jones, Gordon, Norrish and Turnell, Field Experiments, Pye, Insecticides and Fungicides)

Grain legumes

Pea seed-borne mosaic virus (PSbMV). Foliar sprays of the pyrethroid PP321 applied three times between mid-June and mid-July decreased aphid numbers and increased yields of four of five cultivars but seemed not to affect virus spread. Seven days after the second

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spray, when all plots were in flower, there were 90 times as many aphids (mainly *Acyrthosiphon pisum*) on unsprayed plots as on sprayed plots, and 11 days later, towards the end of flowering, there were 30 times as many. At the end of flowering the mean incidence of PSbMV in unsprayed and sprayed plots of cv. Waverex, with 17% seed-borne infection, was respectively 60 and 50%, and the mean incidence in adjacent unsprayed and sprayed plots of the other cultivars, all grown from uninfected seed, was respectively 1·3 and 2·1%. Sprayed and unsprayed plots of cv. Maro yielded similarly (mean, 3·9 t ha^{-1}), but sprayed plots of the other cultivars yielded 18% (Birte), 20% (Progreta), 37% (Vedette) and 55% (Waverex) more than unsprayed plots. (Cockbain and Pu Zuqin with Mr A. J. Biddle, Processors and Growers Research Organisation)

A pea isolate of lucerne vein-yellowing virus (LVYV). Partially purified preparations of LVYV, isolated from peas showing stunting and chlorosis at Rothamsted in 1985 (*Rothamsted Report for 1985*, 124), contained isometric particles *c.* 28 nm in diameter. In immunospecific electron microscopy (ISEM) tests, LVYV reacted strongly with an antiserum to a Dutch isolate of bean leaf roll virus (BLRV) but did not react with antisera to barley yellow dwarf and beet mild yellowing viruses. In host range studies using *A. pisum* and *Myzus persicae* as vectors, LVYV, like British isolates of BLRV, infected crimson clover and pea cv. Dark Skin Perfection but not sugar beet and *Montia perfoliata;* unlike BLRV, it sometimes induced vein-yellowing in lucerne but failed to infect field bean cv. Minden. Symptoms developed in pea seedlings inoculated by aphids that had fed through 'parafilm M' membranes on partially purified preparations of LVYV, but no symptoms developed in lucerne seedlings that were similarly inoculated. (Cockbain; Pu Zuqin, Woods and S. E. L. Roberts)

Fungal invasion of lupins following desiccation. Late ripening of *Lupinus albus* crops limits their wider cultivation. As an aid to drying, desiccants were applied either 99 or 111 days after flowering, to a crop of cv. Vladimir. The crop was harvested on 17 October, six weeks after the first desiccant was applied. Desiccants increased the rate of drying initially to give water contents at harvest, 2–3% less than those of untreated plots. However, some early treatments, especially diquat, applied while the seeds were still green, decreased yields by up to 25%. By harvest, extensive fungal invasion was evident, many of the seeds were discoloured and some fungal mycelium was seen within the pods. All blemishes were most abundant in seeds treated with the higher rate of diquat (3 l 'Reglone' ha^{-1}), especially following the early application. However, all treatments gave more blemished seeds than untreated plots. *Fusarium avenaceum* and a sterile dematiaceous fungus were each isolated from 22% of surface sterilized, extensively browned seeds and *F. culmorum, F. sambucinum* and *F. tricinctum* occurred but less commonly. *Phomopsis, Botrytis, Alternaria* and *Stemphylium* spp. were all isolated regularly from all treatments. (Lacey; Nabb with H. L. Jones, Field Experiments)

Cereal diseases

Eyespot

Epidemiology. The development of eyespot lesions was examined in plots of winter wheat (cv. Avalon) sown on 10 September, 18 October or 4 November and inoculated with wheat (W)-type or rye (R)-type isolates of *Pseudocercosporella herpotrichoides* or uninoculated. Early sowing generally increased the severity of eyespot lesions on plants from uninoculated plots and the incidence of eyespot on leaf sheaths in April was greater in plots inoculated with R-type isolates (82% of shoots) than in plots inoculated with W-type isolates (70% of shoots). Subsequently, the incidence of eyespot declined in plots inoculated with

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R-type isolates and by August 36% of culms had lesions. However, the incidence of eyespot increased in plots inoculated with W-type isolates (82% culms with lesions). These differences may have arisen because of dry weather in May/June when it appeared that the penetration of lesions from the leaf sheaths to the culm was arrested more in plots inoculated with R-type than in plots inoculated with W-type isolates. No such interaction was observed in later-sown plots where lesions developed steadily. In both early-sown and later-sown plots prochloraz plus carbendazim controlled eyespot better when applied at flag leaf emergence (Zadoks growth stage (GS) 37–39) then when applied at the start of stem extension (GS 30–31). (Goulds, Bateman and Fitt)

Late fungicide sprays. Severe eyespot may be forecast most reliably during stem extension (Higgins, Fitt and White, *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* (1986) **93,** 210–220) but forecasts are presently made before stem extension begins, to allow fungicides to be sprayed at GS 30–32. Later forecasting would require spraying when the crop canopy might impede access of the fungicide to the shoot bases. In a field experiment, plots of winter wheat were sprayed with carbendazim or prochloraz at GS 21, 24, 30 or 34–47. Conventional top sprays (but using a knapsack sprayer) were applied at all four times to different plots, and on the last two occasions sprays were applied to the bases of the plants in other plots, simulating spraying with trailed nozzles (Janicke, Grossman and Moser, *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* (1984) **91,** 146–158). In July, eyespot was significantly decreased by carbendazim applied as top sprays at GS 34–37, and by prochloraz applied as top sprays at GS 21 and 24, and as base sprays at GS 30 and 34–37. The eyespot population was partially resistant to carbendazim. (Bateman)

Leaf blotch

Epidemiology. Development of leaf blotch (*Rhynchosporium secalis*) epidemics on winter barley (cv. Maris Otter) and spring barley (cv. Apex) was monitored in plots inoculated with infected straw. There were $3{\cdot}9\times10^5$ condia of *R. secalis* g^{-1} dry weight of straw in samples collected from the plots on 1 November 1985, and there was a mean of $2{\cdot}3\times10^5$ conidia g^{-1} dry weight in ten further samples collected before the end of February. Most spores ($1{\cdot}2\times10^6$) were collected in a sample on 5 March and thereafter the number of spores on the straw declined until there were $1{\cdot}3\times10^6$ conidia g^{-1} dry weight of straw on 3 July when the sampling was stopped. By July there were 10^8 conidia g^{-1} dry weight of leaf, which suggests that leaves had then become the principal source of inoculum. (Davis; Fitt)

Net blotch

Sensitivity to fungicides. When tested, isolates of the net blotch pathogen (*Pyrenophora teres*) from barley harvested in Britain in the early 1970s were very sensitive to triadimenol, an ergosterol biosynthesis inhibitor. However, isolates from crops harvested from 1983 onwards were resistant, tolerating 150μg ml^{-1}. No change was detected in sensitivity to imazalil or propiconazole but there was a drift towards insensitivity to flutriafol (PP 450) and penconazole. (Sheridan)

Take-all

Combining factors known to affect take-all. Sowing date (24 September vs. 18 October); N form (ammonium sulphate vs. 'Nitro-chalk'); N timing (200 kg ha^{-1} in March vs. 40 kg in early February+160 kg at the end of March); autumn N (60 kg ha^{-1} in the seedbed vs. 0); seed dressing ('Baytan' vs. organo-mercury) and soil fungicide (1 kg ha^{-1} nuarimol vs. 0)

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were tested in a half replicate of a 2^6 factorial experiment using a site where the third winter wheat crop was considered to be at high risk from take-all. The mean yield of the experiment was 8·5 t ha^{-1} and the best yield of 9·8 t ha^{-1} was from a late-sown plot with organo-mercury seed dressing, no soil fungicide, 'Nitro-chalk' in the seedbed and again in March only. Eyespot was controlled by prochloraz with carbendazim, 20% of straws had slight sharp eyespot in July and 78% of plants had take-all, although most of this was slight and not expected to affect yield seriously. The late-sown crop yielded more than the early-sown crop (8·78 t ha^{-1} vs. 8·15 t ha^{-1}) and had less take-all in March and April. The divided nitrogen application was associated with less incidence of take-all in April and fewer nodal roots were infected where ammonium sulphate was applied. By July there was no longer an effect of sowing date on slight take-all, although the effect of the divided nitrogen persisted and fewer plants with organo-mercury seed dressing were infected; there was less severe take-all in plots receiving nuarimol. Both nuarimol and ammonium sulphate fertilizer decreased the incidence of sharp eyespot. The relevance of factors shown to affect disease in an experiment where disease, although prevalent, was not severe and seemed not to affect yield greatly is not at this stage clear. (Hornby; Bateman and R. J. Gutteridge)

Soil applied fungicides. Four ergosterol biosynthesis-inhibiting fungicides with different physical properties and behavioural characteristics in soil were compared as soil-incorporated treatments using the small plot method (*Rothamsted Report for 1983,* 122–123). Nuarimol and triadimenol decreased take-all in July after application to the seed bed at 2 kg ha^{-1}. Two compounds which are more volatile, but have similar intrinsic toxicities to the take-all fungus, penconazole and PP 969, had no effect on disease when assessed in July. PP969, which is more polar and mobile in soil water, slightly decreased take-all in April samplings. These results confirm laboratory findings that improved fungicide performance may result from redistribution in soil water, but not in the vapour phase and therefore best control may result from the use of a persistent, moderately polar compound. (Bateman; Hornby and R. J. Gutteridge)

Susceptibility of cereals to take-all. Wheat (cv. Avalon), barley (cv. Panda), rye (cv. Dominant) and triticale (cv. Lasko, cv. Status) were grown as third cereals under two systems of husbandry on two sites. The 'high input' systems provided 40 kg N in February and 160 kg N in April with a full fungicide, insecticide and growth regulator programme, the 'low input' system provided only 120 kg N in April. Take-all was prevalent on both the clay-loam site at Rothamsted and the sandy site at Woburn, and was more severe with the 'low input' system than with the 'high'. At both sites wheat had most infection (take-all rating (TAR) 111) then barley (TAR 83), triticale cv. Lasko (TAR 66), cv. Status (TAR 64) and rye (TAR 34). Triticale cv. Lasko yielded most on both sites and at both levels of inputs with a mean yield of 7·82 t ha^{-1}, then rye (7·12 t ha^{-1}), triticale cv. Status (6·33 t ha^{-1}) wheat (6·16 t ha^{-1}) and barley (6·06 t ha^{-1}). The differences in yield were smaller with the 'high' level of inputs because wheat and barley responded more to the extra inputs. (R. J. Gutteridge; Hornby with Prew, Field Experiments)

Possible biological control agents. Isolates of eight unidentified fungi obtained from wheat roots between 1974 and 1985 inhibited the growth of the take-all fungus on agar. They also had low sensitivity to the ergosterol biosynthesis-inhibiting fungicides currently being investigated for the control of take-all. Most caused slight inhibition of take-all in short-term pot experiments using soil infested naturally or artificially with the take-all fungus, but the effects were not consistent and were always slightly less than the effects of *Phialophora graminicola* and considerably less than the effects of *Phialophora* sp. (lobed hyphopodia), two fungi which are believed to control take-all by competition for root tissue and by inciting

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host responses. However, two of the inhibitor isolates were found to be effective colonizers of wheat roots and one was a weak pathogen. This may be useful for biological control if prior colonization of wheat roots could be achieved. Seed coating with either of the *Phialophora* spp. did not control take-all in short-term pot experiments using naturally-infested soil. (Bateman; R. J. Gutteridge, M. J. White, Henson)

Attempts were made to isolate hypovirulent strains of the take-all fungus, *Gaeumannomyces graminis* var. *tritici (Ggt)*, for possible use as biological control agents. *Ggt, P. graminicola* and *Phialophora* sp. (lobed hyphopodia) were isolated from both field plants and debris from wheat-field soils, using a selective medium (Juhnke, Mathre & Sands, *Plant Disease* (1984) **68,** 233–236). The selective medium was effective only when used in combination with the surface sterilant, silver nitrate. Hypovirulent isolates of *Ggt* were obtained from both sources but at low frequency only (less than 4% of all isolates). (Parsonage; Hornby)

ELISA and the take-all fungus. A report (El Bashaar, Moore & George, *Phytopathology* (1985) **75,** 1363) of the use of ELISA to quantify *Ggt* in wheat roots led to the re-examination of an antiserum prepared in 1968 against two strains (L122 and 2) of *Ophiobolus graminis* (the name then given to *Ggt*). Stock cultures of L122 are now weakly pathogenic and have not been confirmed as *Ggt*. In ELISA the antiserum was not specific to *Ggt,* however, the grouping of test isolates according to strength of reaction with their mycelial extracts (strong: *Ggt, Phialophora* sp. lobed hyphopodia, *P. zeicola)* intermediate: *P. graminicola*; weak: *Cephalosporium maydis*; no reaction: *Fusarium solani* var. *coeruleum* and an unidentified inhibitor of *Ggt*) bears some resemblance to current views on the affinities of these fungi. (Hornby; R. F. White, M. J. White)

Barley yellow mosaic virus (BaYMV). The relationship between BaYMV and its putative vector *Polymyxa graminis* has been demonstrated in three ways: (a) individual resting spores (cystosori) from disease barley plants and checked for freedom from other root-infecting fungi were used to start sand cultures of *P. graminis* on barley roots and some plants subsequently developed symptoms; (b) BaYMV has been detected by ELISA in zoospores obtained from roots of diseased barley plants; (c) zoospores and cystosori produced by a non-viruliferous isolate of *P. graminis* grown in sand culture on roots from infected barley transmitted BaYMV to test seedlings.

Several new barley cultivars or breeders' lines reputed to be resistant to BaYMV were tested in glasshouse experiments. *P. graminis* grew well in their roots but zoospores from them did not transmit virus to susceptible test seedlings. ELISA tests on roots of immune cultivars inoculated with viruliferous *P. graminis* showed that the virus had multiplied little, if at all, in them.

Two samples of BaYMV-infected plants contained virus particles that were not trapped in ISEM tests by antiserum previously produced to an isolate from Bedfordshire. BaYMV was purified from field plants from one of these sites and an antiserum prepared. The new isolate resembles the NM-strain reported from West Germany and is serologically related to wheat yellow mosaic virus which is not known to occur in Britain. (Adams; Jones, Swaby)

Barley yellow dwarf virus (BYDV)

Strain identification. In ELISA using monoclonal antibodies supplied by Dr L. Torrance, MAFF/ADAS, Harpenden Laboratory, and prepared using the Rothamsted isolates B and F, it has been possible to define these isolates more closely. Isolate B is a stable mixture of the PAV- and RPV-like strains, isolate F is a MAV-like strain, and isolate G is a PAV-like

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strain. (These initials signify the specific names of the prinicipal vector aphids, namely *Rhopalosiphum padi, Macrosiphum* (*Sitobion*) *avenae*.)

The monoclonal antibodies were used in indirect ELISAs and supported by direct ELISA and ISEM tests using polyclonal antisera to type strains of BYDV from Central and South America, the near East and the Indian sub-continent. PAV, RPV and MAV-serotypes were identified from most regions but their frequencies differed. Such differences may have an important influence on resistance breeding programmes. (Barker; Forde and Plumb)

Infectivity testing and forecasting. The forecast last year (*Rothamsted Report for 1985*, 125) that BYDV would be much more common in 1986 than 1985 was demonstrated in crops sown in September, but a late harvest meant that most crops were sown later than usual and thus avoided infection. When winter barley cv. Igri was sown on five occasions between 13 September and 23 October and either sprayed or not with cypermethrin (25g a.i. ha^{-1}) on 14 November the 13 September sown plots had 21% infection when not treated with insecticide and 6% when sprayed compared with 3% and 1% when sown on 2 October and 0·5% and 0% when sown on 23 October. Aphids colonizing untreated plots reached a maximum at the end of October and thereafter their numbers decreased rapidly. On plots sown in September there were at least 10 times as many *Rhopalosiphum padi* as *Sitobion avenae*. Despite the excellent agreement between forecast virus risk and field incidence the increased yield given by the cypermethrin spray was significant only for the 13 September (+9·6%) and the 2 October (+8·9%) crops. October–sown crops outyielded the September–sown by 1 t ha^{-1} whether sprayed or unsprayed. (Plumb; Lennon, R. A. Gutteridge with Carter, Entomology)

Testing for virus infectivity was done at eight sites in 1986 and, although data are not complete at all sites the Infectivity Index was much less than the previous year. Consequently the risk of infection appears to be low. The cumulative Index for Rothamsted is 12 compared with 90 last year and a threshold value of 50. (Plumb; Lennon and R. A. Gutteridge)

Potato diseases

Effects of diseases on yield. In comparisons of uniformly infected or healthy populations, stem canker (*Rhizoctonia solani*) decreased yield in October by 6%. Plots with 50% diseased seed yielded only 2% less than plots with all plants healthy because, although in mixed populations yields from diseased seed were decreased by 8%, yields from healthy plants increased by 10%. Plant yields were larger when neighbouring plants were missing but healthy plants benefited from decreased competition more than diseased plants.

Planting seed tubers with gangrene decreased yield by 9% but the yield from mixed populations was only 3% less than from uniformly healthy seed. As with stem canker, yields from diseased plants were suppressed (15%) by the healthy plants which yielded 17% more than in uniformly healthy plots. Also, at wide spacing, plants from diseased seed gave 16% less yield than healthy plants. (Hide; Read, Sandison and Hall)

Effects of *Rhizoctonia solani* from barley on growth and yield of potatoes. In 1983, an isolate of *R. solani* from diseased barley severely damaged potato roots and in mid-season decreased tuber yield by 30% (*Rothamsted Report for 1983*, 126). In 1986, potatoes grown in compost were inoculated with cultures of the barley isolate at two rates. Two weeks after planting, almost all roots of plants in inoculated soil had been pruned off by infection. Further roots grew from the damaged roots and infected plants developed an unusually fibrous root system, but at 12 weeks they had less than half the dry weight of roots of

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uninfected plants. The infection delayed shoot emergence and increase in haulm weight, leaf area and tuber bulking and, at 21 weeks, tuber yields were decreased by 33% (low inoculum) and 63% (high inoculum). (Hide; Sandison)

Resistance of *Helminthosporium solani* to thiabendazole. Since 1968, thiabendazole has become widely used both on tubers at harvest to prevent diseases developing in store and also on seed tubers to improve the health of the subsequent crop. Occasionally, commercial consignments of tubers carrying apparently adequate fungicide have developed severe silver-scurf during storage. In 1986, isolates of *H. solani* were made from treated and non-treated tubers of several sources and grown on 2% malt agar incorporating 5 or 100 μg thiabendazole g^{-1}. Compared with growth on agar without fungicide some isolates produced very small colonies on agar containing 5 μg g^{-1}, whereas the diameter of others was not halved by the higher rate. Further isolates, made from tubers supplied by Dr. G. J. Jellis from breeding clones at the Plant Breeding Institute and by Dr. S. F. Carnegie from early virus tested stem cuttings multiplications at DAFS, were mostly sensitive to thiabendazole. It is not known how quickly resistant isolates become prevalent following annual seed tuber treatment or whether the isolates are also resistant to related fungicides but these observations could explain why thiabendazole sometimes fails to prevent severe silver scurf in store. (Hide; Hall)

Improving the health of home-grown seed potatoes. In 1985, rogued plots of King Edward potatoes treated with phorate (1·7 kg ha^{-1}) at planting and foliar sprays of pirimicarb (0·14 kg ha^{-1}) yielded tubers which, on growing on in 1986, had about 8% potato virus Y (PVY) whereas plants protected with additional sprays of mineral oil (7 1 ha^{-1}) plus cypermethrin (40 g a.i. ha^{-1}) either until roguing was completed (three weeks from emergence) or throughout the growing season had only 2% infected. Plots of the more PVY resistant cv. Maris Piper had only 0·5% infected tubers.

Potato leafroll virus occurred infrequently in all treatments in both cultivars except in one of the plots of Maris Piper treated throughout the growing season with oil plus cypermethrin; this plot had 14% infected tubers. However, very (R_2) and extremely (R_3) insecticide-resistant forms of *M. persicae*, the main vector of leafroll, colonized this experiment and were most abundant on the plots sprayed repeatedly with cypermethrin and oil. (Gibson; with Harrington, Entomology and ffrench-Constant, Entomology)

Potato virus diseases at Rothamsted. When counts were made in late June, plots planted with Pentland Crown seed grown at Rothamsted in 1985 were free from virus infection. Similar plots of King Edward had 0·02% PVY, and Désirée had 0·01% PVY and 0·15% leafroll. In 1986, aphids (*M. persicae*) were scarce until early August and no current season infection with PVY was detected in crops planted to provide seed for 1987. A model for prediction of PVY infection is described on p. 87 (Govier)

Viruses

Beet cryptic viruses (BCVs). Double-stranded (ds) RNA extracted from purified BCV was copied into cDNA and cloned into pUC9. Clones were identified which correspond to three (RNA1, 3 and 4) of the four dsRNA components of BCV. These did not hybridize to each other or to RNA2, suggesting that there is no significant sequence homology between the four dsRNA components. RNA extracted from 20 beet plants was analysed by northern blotting using the cDNA clones as probes. Fifteen plants were found to contain RNAs 3 and 4, and nine of these also contained RNA1. These results are compatible with the occurrence in these plants of two different viruses. The sensitivity and specificity of the cDNA hybridiza-

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tion assay was greater than that of ISEM in the detection of BCVs. (White; with Antoniw, Biochemistry and Drs H. J. M. Linthorst and J. F. Bol, University of Leiden)

Fastidious prokaryotes

Sumatra disease of cloves. This disease is caused by an unnamed Gram-negative bacterium which has been cultured axenically and is now routinely grown on a buffered Casamino acids medium containing ferric ammonium citrate.

Polyclonal antisera raised to the Sumatra disease bacterium (SDB) gave strong cross-reactions with *Pseudomonas solanacearum*. Absorption of the anti-serum with *Ps. solanacearum* resulted in a very low specific titre suggesting a strong serological relationship between the two organisms. This relationship has been further investigated using monoclonal antibodies, DNA homology, fatty acid composition and conventional bacteriological tests.

Monoclonal antibodies have been produced (at AFRC Monoclonal Antibody Unit, Institute of Animal Physiology, Babraham) to SDB and *Ps. solanacearum*. Clones were screened for their ability to discriminate between SDB and *Ps. solanacearum* by ELISA. First attempts using formaldehyde fixed bacteria as the antigen failed to produce any specific antibodies but alternative antigen preparations of these bacteria are now being investigated.

The percentage guanine+cytosine content of DNA from strains of SDB was found to be identical with that of *Ps. solanacearum*. A qualitative investigation of the homology between DNA from SDB and *Ps. solancearum* was made using dot blot hybridization between membrane bound DNA from these and several other bacteria and a radioactively labelled probe prepared from SDB. The probe DNA hybridized most strongly to itself, less strongly to *Ps. solanacearum* and weakly to *Ps. fluorescens* and *Ps. cepacia*. No hybridization was detected between the SDB probe and the Pierce's disease bacterium or *Rhizobium phaseoli*. (S. J. Roberts; Ambler)

Aerobiology

Dispersal of *Rhynchosporium secalis* conidia from barley debris and barley leaves. Simulated rain (mean drop diameter *c.* 1, 2 or 3 mm) was allowed to fall for 10–15 min on to barley debris or detached leaves infected with *R. secalis*. The leaves were supported on a mesh through which run-off water drained and the debris was supported on a rigid surface on which run-off water collected. The numbers of *R. secalis* conidia and spore-carrying splash droplets collected by horizontal samplers decreased with increasing distance from and increasing height above the sources. Horizontal gradients and vertical profiles of spore deposition were steep, with half-distances of 5–8 cm and 2–9 cm respectively. Gradients and profiles of spore-carrying droplet deposition were almost as steep, with half-distance of 8–10 cm and 5–10 cm respectively. Incident drops 3 mm in diameter dispersed more conidia and more spore-carrying droplets than incident drops 1 or 2 mm in diameter. The droplet size category containing the greatest proportion of the spore-carrying droplets dispersed by 3 mm drops was 200–400 μm, whether the source was free-draining barley leaves or barley debris plus run-off water. The greatest proportions of spores were carried in droplets in the size categories 200–400 μm and 400–600 μm for leaves or debris respectively. (Fitt; Creighton with M. E. Lacey, McCartney and Walklate, Physiology and Environmental Physics)

Airborne microorganisms associated with domestic waste disposal. A three year programme of bimonthly air sampling at 16 domestic waste transfer sorting facilities and on

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landfill sites was completed, using two sampling methods; multistage liquid impingers (MSLI) and aerosol monitors collecting on to polycarbonate membrane filters. Aerosol monitors were most useful when there was no power source, e.g., on landfill sites, in the cabs of cranes and bulldozers moving refuse, and for personal sampling, but the MSLI was better for area sampling.

Concentrations (colony forming units (CFU) m^{-3}) of airborne microorganisms (bacteria isolated at 37°C and fungi at 25°C) were greatest where refuse was pulverized and sorted (bacteria $4{\cdot}3\times10^4$–$5{\cdot}9\times10^5$, fungi, $1{\cdot}5\times10^5$–$3{\cdot}3\times10^6$), next to tipping bays where refuse was unloaded from collection lorries (bacteria, $3{\cdot}8\times10^4$–$4{\cdot}9\times10^5$; fungi, $2{\cdot}4\times10^5$–$7{\cdot}6\times10^6$) and where refuse was removed from bunkers by grab cranes (bacteria $1{\cdot}1\times10^5$–$7{\cdot}8\times10^5$; fungi, $1{\cdot}2\times10^6$–$1{\cdot}1\times10^7$). Similarly at landfill sites, airborne microorganisms were most numerous when refuse was being spread and buried. Most bulldozer cabs were air conditioned but when drivers left their cab doors open concentrations of airborne bacteria and fungi inside were up to 100 times greater than when the doors were kept closed. At all sites most microorganisms were present in the summer.

The predominant bacteria were species of *Bacillus* but Gram-negative bacteria were grown from more than 90% of air samples, often at concentrations exceeding 10^4 CFU m^{-3}. The most common Gram-negative bacteria were *Pseudomonas* spp. (59·3% of the total) and *Klebsiella/Enterobacter* spp. (21·4%). *Serratia* spp. and *Escherichia coli* were infrequently isolated. *Penicillium* spp. were the most commonly isolated fungi (81% of samples) with yeasts in 58% of samples and *Aspergillus fumigatus* in 40%. *Penicillium* spp., on average, exceeded 10^4 CFU m^{-3} in the respirable fraction of MSLI samples collected throughout the year. Average counts of *Aspergillus* spp. and *Cladosporium* spp. in the respirable fraction only exceeded 10^3 CFU m^{-3} between July and September.

The numbers and types of fungi and bacteria isolated suggest that there could be a health risk for some individuals during waste disposal. Thus, every effort should be made to avoid unnecessary exposure of workers, especially when refuse is being pulverized or redistributed on landfill sites. (Lacey; Higgins and Crook)

The detection of airborne allergens. The efficiencies of possible methods of collecting airborne allergens were compared in a large wind tunnel using aerosols of fungus spores. Eleven collection and handling methods were used with eight different air sampling devices. Most efficient was a large-volume electrostatic precipitator which collected particles into liquid.

In an outbreak of occupational asthma in a sugar beet factory, allergy to sugar beet protein was identified but symptoms could have been exacerbated by the many airborne gram-negative bacteria also present in aerosols produced by slicing machines and conveyors. Exhaust ventilation decreased concentrations of airborne particulate matter from 3·58 mg m^{-3} to 0·61 mg m^{-3} during the course of a year and also decreased incidence of respiratory symptoms.

In a large bakery airborne bacteria and fungi usually occurred in similar numbers to other indoor environments but both were more numerous in areas where the raw ingredients were handled and mixed. Airborne particulate concentrations in the breathing zones of workers ranged from less than 1 mg m^{-3} in packing areas to 37·6 mg m^{-3} in mixing areas, almost four times the HSE recommended threshold for occupational dust exposure, and it was associated with sneezing and rhinitis in workers despite respiratory protection. (Lacey; Crook, Williamson and Wilson with Dr M. D. Topping and Miss H. W. Forster, (Occupational Medical and Hygiene Laboratories, HSE, Cricklewood), Dr A. J. Newman Taylor and Dr R. D. Tee (Cardiothoracic Institute, Brompton))

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Staff and visiting workers

It is with much sadness that we report the death of Dr P. H. Gregory, FRS, who was Head of Plant Pathology from 1958–1967 and an Honorary Scientist in the Department. He will be greatly missed. Alex Bainbridge retired and Mary White left. Tracy Feekins was replaced by David Marshall during her maternity leave. Steven Roberts, David Ambler and Kathryn Boorer were appointed. Timothy Jones and Guido Herrera joined as Ph.D. students. Members of the Department visited Kenya, West Germany, France, India, People's Republic of China, Israel, Sweden, Denmark, Mexico, USA, Madeira, United Arab Emirates, South Yemen and Switzerland during the year in furtherance of research contacts and to participate in meetings. Visitors who came to work in the Department for different periods came from People's Republic of China, India, Pakistan, New Zealand, Mauritius, Zanzibar, Chile and France. John Lacey and David Hornby continued as Chairmen of ISPP committees and several other members of the Department play an active role in scientific societies. We are grateful to the Overseas Development Administration, MAFF, Potato Marketing Board, Health and Safety Executive, Bayer, BASF and the Hills Bequest for financial support.

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