ROTHAMSTED RESEARCH

Rothamsted Experimental Station Report for 1987

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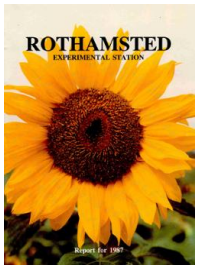

Crop and Environment Protection Division

T. Lewis

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INTRODUCTION

The year has been one of considerable reorganization within the Division to ensure that the best use is made of diminishing staff resources and that the work on aspects of crop protection at Rothamsted and Long Ashton is effectively integrated.

In September the Entomology and Nematology Departments were merged under the Headship of B.R. Kerry thus bringing together two well-established and historic Departments, each of which has made very important contributions to pest biology and control nationally and worldwide. Plans are well advanced to house the new much larger Department in the Daniel Hall Building and to integrate the specific knowledge and expertise characteristic of these separate branches of zoology to produce even more effective and innovative research.

On a wider scale the three Crop Protection Departments at Rothamsted have been joined by the Crop Protection Department at Long Ashton Research Station (LARS) to form a single Crop and Environment Protection Division spanning these two main component sites of the Institute of Arable Crops Research. This arrangement has great potential for bringing together complementary skills especially related to plant pathology, pesticide chemistry and spray application methods. To ensure the integration of programmes and staff at all levels, the Head of Division and four Heads of Departments now hold monthly management meetings, and all staff have been assigned to at least one coordination group to encourage a free flow of ideas, avoid possible duplication of effort, and to identify possible sources of funding. The Division has an excellent record of attracting external funds to date, and there is good reason to believe that the new arrangements will enhance its prospects.

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ENTOMOLOGY AND NEMATOLOGY DEPARTMENT

The new Department was formed on 1 September 1987 following further cuts in government funding for arable crops research and changes in priorities within the Agricultural & Food Research Council (AFRC). Over the past four years these policies have resulted in the loss of 46% of Entomology and Nematology staff and much expertise. The Entomology Department was established by A.D. Imms in 1922 and the Nematology Department began at Rothamsted in 1947 with the arrival of T.G. Goodey; both departments had distinguished scientific reputations and made significant contributions to the control of pests in arable crops. The core of staff remaining within the merged department is very capable of continuing these traditions but can no longer investigate as wide a range of problems as in the past. Some losses have been made up by outside funding which this year formed 15% of the budget and supported 25% of the scientific staff.

Research is directed toward the maintenance of effective pest control by methods which limit pesticide applications and minimize effects on the environment. In the future we expect to expand our interests in farmland ecology, the development of new methods of diagnosing pest problems and novel methods of pest control. The Department has been reorganized into four research groups which should enable closer collaboration between entomologists and nematologists and ensure that the research programme meets the above priorities; this year's research is reported below in line with the new structure.

Pest and environment management

The main emphasis of research within this group is to develop efficient pest management strategies which are environmentally acceptable. In developing such strategies the use of resistant cultivars and natural enemies to enable a reduction in pesticide usage have been investigated. Research has continued on the effects of changing agricultural practices, such as straw incorporation, on pest control, and potential pest problems of new break crops have been studied. The role of honeybees in the pollination of these break crops and the effects of diseases on colony vigour continue to be important topics of the Department's research. These and other aspects of the group's work are reported here.

Aphid parasitoids and aphid populations. Research on the behaviour of aphid parasitoids, aimed at developing ways of enhancing parasitoid impact on pest aphid populations, has continued. Host preference trials using the polyphagous parasitoid *Aphidius ervi*, which attacks cereal, pea and nettle aphids, indicated that genetic factors may play a role in host recognition by egg-laying females. Few females attacked nettle aphid, *Microlophium carnosum*, if they, and both their parents had been reared on pea aphid, *Acyrthosiphon pisum*. However, if the male parent had been reared on nettle aphids, females attacked them to the same extent as they did pea aphids even though they, and the female parent had been reared on pea aphids. (Powell and Wright)

Work on behaviour-controlling chemicals (semiochemicals) has concentrated on the effect of aphid honeydew as a searching stimulant for parasitoids. Video recording was used to study behavioural reponses of parasitoids to honeydew solutions applied to filter paper discs. Both primary parasitoids and hyperparasitoids of cereal aphids collected from the field showed strong responses: areas treated with cereal aphid honeydew were searched for much longer periods than untreated controls. Both females and males responded but the female response was generally stronger. Intensification of searching behaviour after encountering honeydew would increase a female parasitoid's chances of locating host aphids and a male's chances of finding unmated females. Searching stimulants are important for preventing or delaying

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dispersal of adult parasitoids from crops during periods of low aphid density, especially at the beginning of pest infestations when parasitoids can significantly reduce aphid population growth. (Powell and Budenberg)

Estimating the potential side-effects of autumn pyrethroid applications to cereals. The effects of deltamethrin applications, either in mid-October or early November, on the polyphagous predator fauna of a winter wheat crop was assessed in plots bordered by polythene barriers. The abundance/activity of staphylinid and carabid beetles, as measured by pitfall traps, was depressed for three to four weeks following application. Spider abundance/activity was greatly reduced following application and remained depressed by comparison with that in control plots until the following spring. In the case of some spiders, notably the abundant *Oedothorax* species, these effects persisted until June. Aphid populations remained low on the crop and no effects of reduced polyphagous predator activity on the numbers of aphids were detected. (Ashby, Wright, Carter and Powell with Dr D.A. Cooper and D.F. Powell, Ministry of Agriculture Fisheries & Food, MAFF Harpenden Laboratory).

Pests of break crops

Lupins and sunflowers. The insect fauna occurring on plots of sunflower and lupin at Rothamsted and elsewhere in southern and eastern England was monitored for a second year. The study included four species of lupin grown at Rothamsted *Lupinus albus, L. luteus, L. angustifolius* and *L. mutabilis*.

The insect species observed on the crops were largely the same as those found in 1986. The numbers of aphids were generally low but *Brachycaudus helichrysi* again heavily infested the flower-heads of some sunflower varieties. *B. helichrysi* and *Macrosiphum euphorbiae* formed small colonies on lupin and, except on *L. angustifolius* which appeared to be more susceptible, infestations of the lupin aphid *M. albifrons* were also small. *L. albus* and *L. luteus* suffered more feeding damage from the adult pea and bean weevil *Sitona lineatus* than did the other lupin species, however there was generally little difference between the species in the occurrence of insect damage. Slug damage to lupins and sunflowers was more widespread than in 1986, particularly at the seedling stage, and *Arion fasciatus* and *A. silvaticus* were identified from lupin and sunflower plots respectively. (Ferguson)

Linseed. For the first time plots of linseed were also monitored for the occurrence of insects. Few insect species were found. However, thrips, mainly *Thrips angusticeps*, were present from the seedling stage throughout the life of the crop, infesting terminal buds, leaf axils, flower buds, flowers and seed-heads. They caused distortion and yellowing of leaves and may have been responsible for the loss of flowers, about 20% of which failed to develop further than buds. These aborted buds had damage to their reproductive structures which could have been caused by thrips. *Cnephasia interjectana*, the flax tortrix moth, infested a small number of terminal buds in May, and in September the large flax beetle *Aphthona euphorbiae* (a flea beetle) was found at all sites. (Ferguson)

Oilseed rape pod midge. The brassica pod midge *Dasineura brassicae*, an important pest of both winter and spring oilseed rape, causes premature scattering of pods and loss of seed. The phenology of its emergence from overwintering sites, previously sown with spring oilseed rape, and its subsequent infestation of spring rape crops has been studied over the past three years. Adults emerged from the overwintering sites from mid-May until early July, but infestation of spring rape did not begin until late June. Two generations occurred on spring

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rape. Mature larvae dropped from the pods almost daily from early to mid-July until mid-August to early September to form cocoons in the soil. A small proportion of these larvae developed into pupae and emerged as a second generation of adults to lay further eggs in the crops. Larvae from these eggs dropped to the soil to diapause within cocoons. (Williams and Martin)

Pollination studies. The effect of insect pollination on plant development and seed yield of winter oilseed rape, cultivar Jet Neuf, has been investigated by comparing plots caged with a honeybee colony, plots caged to exclude insects, and plots uncaged and 'open-pollinated' by naturally occurring insects. Plants in the bee-pollinated plots finished flowering earlier, showed more advanced pod growth, and were shorter than those in the plots without bees. Pods from the plots with honeybees contained more seed post-flowering than those from plots without honeybees but the proportion of them that grew into mature seeds for harvest was determined by water availability during seed growth. (Williams and Martin)

Studies on pollination requirements are continuing on lupins and have begun on linseed. (Williams, Richards, Ferguson and Martin)

Nematicide trials with oilseed rape. Substantial yield increases have been obtained by nematicide treatment of 'single-low' cultivars of oilseed rape growing in soil infested with *Heterodera cruciferae*, the brassica cyst nematode (see *Rothamsted Report for 1986*, 107-108). Cultivars which are low in both glucosinolates and erucic acid ('double-low' cultivars) have recently become available and the subsidies applied to this crop usually make them more profitable than single lows. Two double-low cultivars (Ariana and Lirabon) were grown alongside two single-low cultivars (Bienvenu and Mikado) in a field near Dover, Kent, with an infestation of *H. cruciferae* averaging 48 eggs g^{-1} soil. A broadcast treatment of aldicarb at 4 kg a.i. ha^{-1} was applied immediately before drilling to half of the plots; the other half were left untreated to act as controls. Yields of the double-low cultivars averaged 1.44 and 1.71 t ha^{-1} in the untreated and treated plots respectively, whereas yields of the single-low cultivars averaged 0.94 and 2.41 t ha^{-1} in untreated and treated plots respectively. A period of nine days was allowed between harvest of the single- and double-low cultivars, because the double-low cultivars mature later. During this period there was heavy bird damage (linnets, skylarks, sparrows) and some pod shatter, so that yields of the double-low cultivars were undoubtedly depressed relative to those of the single-low cultivars. Despite this, the double-lows yielded substantially more than the single-lows in the untreated plots. It seems likely, then, that double-low cultivars of oilseed rape are more tolerant of cyst nematode attack than single-low cultivars. At least part of this better yield is due to improved plant survival: plant population densities at harvest averaged 154 m^{-2} for both types of cultivar in nematicide treated plots but averaged 92 and 132 m^{-2} for single- and double-low cultivars respectively in untreated plots. There were no differences between cultivars in final nematode population density, which averaged 145 and 162 eggs g^{-1} soil in untreated and nematicide treated plots respectively. (Evans and Russell)

Integrated control of potato cyst nematodes. The multidisciplinary intensive potato experiment at Woburn (see p. 28) has again shown that susceptible, intolerant potatoes (cv. Désirée) can be grown without loss of yield or quality in a four-course rotation, in land infested with potato golden cyst nematode (*Globodera rostochiensis*), if the seedbed is treated with an effective nematicide (oxamyl at 5.6 kg ha^{-1}) just before planting.

In a repeated study of tolerance of *G. rostochiensis* by Maris Piper, Pentland Crown, Désirée and Pentland Dell potatoes at Woburn, Maris Piper again exhibited tolerance to attack but it was less marked than in 1985 and there was little difference in tolerance between the other

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cultivars, whereas in 1985 Pentland Crown was less tolerant than Désirée or Pentland Dell. The results show that the expression of tolerance in an individual field is influenced by environmental conditions. Nevertheless, new potato genotypes are screened by plant breeders to eliminate intolerance to the potato pale cyst nematode (*G. pallida*), now the commoner of the two species in Britain. In 1987 we assessed the effects of halving the amount of oxamyl applied to the seedbed in spring on the control of damage due to *G. pallida* in fairly heavily infested silty, sandy and peaty loam soils, in which tolerant and partially resistant cultivars or clones were grown. The tuber yields of susceptible Désirée and Romano and partially resistant Santé, Glenna, Fingal, Morag, Valiant, Caxton and clones ZB 35-29 and ZB 35-177 were sometimes increased as much by 2.8 as by 5.6 kg oxamyl ha^{-1} (approved commercial dosage). The results suggest that it may be possible to control *G. pallida* with less nematicide than usual when tolerant, partially resistant potatoes are grown. It has yet to be determined if nematode increase was adequately controlled and if such integrated control of *G. pallida* can succeed in a wider range of soils over a number of years.

The reliability of resistance to eight populations of *G. rostochiensis* and/or partial resistance to 28 populations of *G. pallida* in Santé, Glenna, Fingal, Morag, Valiant and clone ZB 35-29 was assessed in comparison with the fully susceptible Désirée. Valiant and ZB 35-29 were susceptible to *G. rostochiensis*, the other cultivars were resistant. Of the 28 *G. pallida* populations, appreciable susceptibility was displayed to 23 by Valiant, to 17 by Morag, to 11 by Fingal, to 4 by Glenna, to 2 by Santé and to 1 by ZB 35-29.

Localized removal of the soil compaction resulting from frequent cultivation of potatoes at Woburn increased the yield response to oxamyl in Désirée and Cara potatoes by some 40% in sandy loam heavily infested with *G. rostochiensis*. Clearly the full yield benefit of an effective nematicide treatment can only be obtained when soil conditions favour good development of the crop. (Whitehead, Nichols and Webb)

Tolerance trials with potatoes and potato cyst nematodes

Assays for tolerance. A field trial design using single plants as plots, 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; *Rothamsted Report for 1986*, 109). Two experiments of this type, using eight cultivars and clones, were done in 1986. In the first, the trial area was divided into four and aldicarb applied at 0, 1, 2 or 4 kg a.i. ha^{-1} and incorporated by rotavation. Ten tubers of each cultivar were planted in each area on 6 May using a randomized block design with standard plant and row spacings. In the second experiment, ten tubers only of each cultivar were planted (on 7 May) but four guard plants (cultivar Désirée) were planted around each test plant, again using standard plant and row spacings. Tubers were harvested from individual plants of both experiments on 20 September and the clones and cultivars ranked for tolerance of nematode attack. Ranking was firstly on the basis of the mean yield of the ten plants growing in untreated soil in both experiments (average population density 150 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 first experiment only. On the basis of yield in untreated soil Maris Piper was the most tolerant to nematode attack, with Vantage and clone 13350 inseparable in second place. However, on the basis of the regression coefficient for cultivar yield against mean yield of all cultivars, Vantage was the least tolerant and clone 13350 the most tolerant. This difference arises because Vantage has a very large yield potential (more than 2.5 kg per plant with 4 kg ha^{-1} of aldicarb) which is much reduced by nematode attack, whereas the much smaller yield potential of clone 13350 (about 1.2 kg per plant with 4 kg

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ha^{-1} of aldicarb) is much less reduced by nematodes: Vantage and clone 13350 both yielded about 0.8 kg per plant in the absence of nematicide. (Evans and Russell)

Physiological ageing of seed tubers and tolerance. Frequent growing of potato cultivars such as Maris Piper, with the H_1 gene for resistance effective against *G. rostochiensis*, can select *G. pallida* from mixed field populations of potato cyst nematodes. Control of *G. pallida* with nematicides is less effective in some soils than control of *G. rostochiensis*, probably because *G. pallida* takes longer to hatch after planting, and nematicides applied before planting fail to persist long enough in soil to exert control of this species. This delay of hatching suggests that a rapidly growing potato crop would establish more effectively than a slower growing crop in a *G. pallida* infested field. The speed of crop establishment can be enhanced by conditioning potato seed in warm chitting houses before planting. Preliminary results suggest that this could be beneficial for some cultivars grown on *G. pallida* sites. (Haydock)

Integrated control of stem nematodes. Resistance to stem nematodes (*Ditylenchus dipsaci*) in lucerne and red clover is often unreliable in our experience and more genes for resistance need to be incorporated in commercial cultivars. Resistance to 'lucerne race' stem nematode was found in 10 of 23 species of *Medicago* and resistance to 'red clover race' stem nematode in 14 of 42 species of *Trifolium*, all kindly supplied by the Welsh Plant Breeding Station. Under a collaborative project with Institut National de la Recherche Agronomique, perennial species of *Medicago* will be screened for resistance to a mixture of French and English populations of 'lucerne race'. Useful resistance will be incorporated in the breeding programme to improve resistance to this pest, an important limiting factor to expansion of this alternative crop in Britain. A glasshouse experiment in 1987 with 11 French and 11 English populations of 'lucerne race' stem nematode suggests they they all come from the same gene pool, although there are differences in virulence between populations. (Whitehead and Nichols)

Varroa and virus infections of honeybees. The Asiatic bee mite *Varroa jacobsoni* is now established in colonies of *Apis mellifera* on every continent except Australasia. The mite is considered a serious threat to world beekeeping and the loss of many colonies of *A. mellifera* have been attributed to infestation with the parasite. However, its effect on colonies in different areas of the world is variable and still poorly understood.

Previous work established that acute paralysis virus (APV) was a major cause of both adult bee and brood mortality in severely infested honeybee colonies in Southern Germany. Recent work in collaboration with the Aristotle University, Thessaloniki extended investigations of the incidence of pathogens in mite-infested colonies to another geographic and climatically very different area. Dead adult bees were collected from beneath the entrances to 15 colonies at two different sites near Thessaloniki, on six occasions during the period March to November 1986. Ten colonies at one site were left untreated and the mite and bee populations allowed to develop normally. The five colonies at the other site were treated with an acaricide in spring which reduced, but did not eliminate, the mite population during the period of observation.

Six viruses were detected by immunodiffusion in the extracts of dead bees from the sampled colonies. Bee virus X (BVX) was present in almost all colonies from late spring to mid summer but none was detected in October or November. In Britain this virus occurs mainly in the overwintering population and rarely persists after June. As BVX infects adult bees only by ingestion it is unlikely that *V. jacobsoni* was directly associated with its transmission. Acute paralysis virus (APV) was detected in 70% of the sampled colonies and occurred even when mite populations were very low. APV incidence increased from May to July as previously

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observed in mite-infested colonies in Germany. However, in contrast to these earlier observations APV infection declined during the autumn. The only other virus commonly present in colonies was a strain of Egypt bee virus, most closely related to a Japanese isolate (JEBV). This virus occurred in some colonies throughout the sampling period but its peak incidence was in late autumn. JEBV has only recently been identified and is not yet fully characterized, but its detection in samples of honey bees received from several countries suggests that, like APV, it is also associated with *V. jacobsoni*. The incidence of the three other viruses detected, black queen-cell virus, chronic paralysis virus and cloudy wing virus, was similar to or less than that in uninfested honey bee colonies in Britain. (Ball and Allen)

Insect-caused false alarms from automatic fire detection systems. The cereal thrips, *Limothrips cerealium* Halliday, has been shown to cause over 90% of all false alarms triggered by insects from smoke detectors installed in public and private buildings. The problem is confined mainly to rural areas, where cereals are grown, which are mostly serviced by retained (part-time) firemen. The Home Office has instigated a programme of research into the prevention of false alarms because of the rising costs of unnecessary turnouts to local authorities and to the employers of retained firemen.

In summer the winged female thrips from ripening cereals find crevices in which to overwinter. They accumulate on cereals until the weather is hot and dry when mass flights occur and many individuals fly indoors, eventually to enter and trigger smoke detectors. Hence, false alarms triggered by thrips tend to be concentrated over only a few days in each summer. For example, during a four-day period in July 1985 at least 70% of all fire service call-outs in Suffolk were due to thrips. The same pattern was repeated in July 1986. Over 70% of all fire brigades in Britain have attended false alarms caused by insects since 1983 and at least 30% consider insects to be a problem despite thrips-proof detectors having been available for at least four years.

A laboratory test has therefore been developed to ascertain the sensitivity of different types and models of detector to insects. Design features likely to reduce a detector's susceptibility have been identified. However, as many detectors are unlikely to be replaced for ten to twenty years, the protection of insect-sensitive models using insecticides or insect-repellants is being investigated. (Cuthbertson)

Heavy metal uptake by soil invertebrates. Investigations continued on the uptake of heavy metals by soil-dwelling invertebrates at dredged material disposal facilities in the United States. Extensive sampling of invertebrates, using pitfall traps and formalin vermifuge, was done at several sites: Times Beach (Buffalo, New York), Black Rock (Bridgeport, Connecticutt), Ottawa Minespoil (Ottawa, Illinois) as well as a reference site at Grand Island (Buffalo, New York). Samples were returned to the laboratory at Rothamsted for identification; individuals were sorted into taxonomic groups and, where samples were sufficiently large, metal analyses were undertaken.

In the laboratory, dredged material collected from each of the sites was used to study uptake of heavy metals by the earthworm *Eisenia foetida* after 28 days' exposure to the dredged materials. The results of these uptake studies will be compared with metal concentrations measured in other invertebrates colonizing the sites to ascertain the relevance of laboratory uptake studies in estimating metal movement into the tissues of organisms living there.

At Times Beach, in addition to the soil micro- and macro-invertebrates (including native earthworms), samples of dredged material (surface and deep layer), vegetation (living and leaf litter) and vertebrates were also collected by other investigators and analysed for heavy metal concentrations. A collation of the data gathered at Times Beach should enable an analysis to be made of heavy metal movement within an ecosystem developing at a confined disposal

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facility. (Stafford and Ashby, with McGrath, Cosimini and Fearnhead, Soils, and Simmers, Rhett and Brown of Waterways Experimental Station, Vicksburg, Mississippi)

Pest monitoring and forecasting

Pest monitoring remains an important priority as a means of ensuring that pesticide applications are not applied unnecessarily or at the wrong time. The Department's extensive database on the numbers of aphids caught in suction traps continues to be used to develop forecasts of virus spread and to model the buildup of aphids in crops. Also, data from the light trap monitoring scheme continues to be analysed to determine whether moths can be used as indicators of environmental change, especially changes in agricultural practice and countryside management.

Aphid monitoring. The network of 22 suction traps throughout Britain continued to provide data on the distribution and aerial abundance of pest aphids. The basic data and an interpretation, including forecasts, published weekly in the *Aphid Bulletin* and *Aphid Commentary* were distributed by mail and viewdata to 300 individuals and organizations in the industry. (*Rothamsted Report for 1983,* 89). (Tatchell, Alderson, Dupuch, Payne, Parker, D.K. Riley and Taylor)

Cereal aphids in summer. A forecasting scheme being developed for aphid control must take account of the interaction between yield expectation and numbers of aphids on final yield. Results from the multidisciplinary wheat experiment at Rothamsted, which ran from 1978/9 to 1983/4, and observations from the Cereal Aphid Monitoring Scheme, RISCAMS (*Rothamsted Report for 1986,* 86) suggested that inputs, such as nitrogen and fungicides which kept crops green for longer, encouraged larger numbers of aphids and yield loss was greater. A field trial was designed to give a range of yields by manipulating nitrogen and fungicide applications in the presence and absence of aphids. Pirimicarb was applied at regular intervals during the summer to maintain aphid-free plots. Nitrogen and fungicides, separately and as an interaction, had a significant effect on yields, which ranged from 5.99 to 8.32 t ha^{-1} but no effect of aphicide was detected as aphid populations, predominantly *Metopolophium dirhodum*, were too small (10 per shoot). Initially more aphids were present in plots receiving no fungicide and the highest nitrogen levels, but these differences disappeared quickly. (Carter and Zhou)

Cereal aphids in autumn. An analysis of the proportion of female *R. hopalosiphum padi* reproducing during barley yellow dwarf virus (BYDV) infectivity tests, from different sites in England, suggested that the proportion of alate exules present in autumn varied annually and regionally. An estimate of the number of alate exules at this time would give a more accurate forecast of BYDV risk and reduce unnecessary insecticide applications.

Host plant choice tests to distinguish *R. padi* gynoparae, which colonize bird cherry (*Prunus padus*), and alate exules, which colonize cereals and grasses, again showed that the latter were replaced almost exclusively by the former during September. Consequently populations on barley sown in September remained below 0.1 per plant. (Tatchell and Carter)

Potato virus Y (PVY) risk assessment in home-saved seed. An unusually large number of *Myzus persicae* flying in early August 1986 gave rise to concern over the forecast of low PVY risk in 1987 home-saved plantings, which is based on aphids caught until the end of July. However, virus incidence in the most susceptible cultivar, King Edward, grown at

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Rothamsted was low (see report for Plant Pathology Department pp. 68-79) and there have only been isolated reports of problems. Wet and cool weather in 1987 resulted in few aphids and the risk of an unacceptable level of PVY in home-saved seed for planting in 1988 is considered low for all cultivars in all regions. (Harrington, with Gibson and Govier, Plant Pathology)

Resurgence of *Myzus persicae* in cypermethrin treated seed potatoes. A massive build-up of *M. persicae* occurred in August in plots of cv. Maris Piper treated every two weeks with a cypermethrin/oil mixture and with pirimicarb to control virus spread. Prior to the resurgence most aphids were moderately resistant (R_1) to insecticides but those found in August were mostly highly resistant (R_2 or R_3). No such buildup occurred in plots treated only with pirimicarb, neither was there a resurgence of *Macrosiphum euphorbiae*, present at the beginning of the season, which lacks resistance to insecticides. There was no evidence that the cypermethrin/oil mixture stimulated aphids to reproduce and it is suspected that the mixture greatly reduced natural enemy populations, allowing uncontrolled multiplication of insecticide resistant forms. (Harrington with ffrench-Constant and Devonshire, Insecticides and Fungicides and Miss E. Bartlet, M.Sc. student, Southampton University)

Cold hardiness of *Myzus persicae*. *Myzus persicae* was found to die under laboratory conditions at temperatures considerably above their supercooling point. Acclimatization at 10°C and 5°C did not affect supercooling but lowered the lethal temperature. Freezing of leaves during feeding did not increase mortality. The lethal temperatures found in the laboratory were similar to those at which high mortality is found in the field and render supercooling point determination meaningless in ecological terms.

The time of the first record of *M. persicae* in suction trap samples was correlated with January and February temperatures except in the west of England and Wales. Further north December and January temperatures became more important in determining the time of dispersal. (Harrington with Dr J.S. Bale and Mr M.S. Clough, AFRC research grant with Leeds University)

Forecasting incidence of virus yellows in sugar beet. A revised forecast of the incidence of virus yellows in sugar beet has been derived. The forecast uses a formula accounting for 89% of the variance in virus incidence and is based on the prevalence of virus in the previous year, the number of days in January and February when the grass minimum temperature falls below 0°C, and the time of first record of *M. persicae* in the Brooms Barn suction trap. A preliminary formula usable at the end of February before the first aphid flight accounts for 66% of the variance in virus incidence. (Harrington with Dewar, Broom's Barn and George, RESCU)

Environmental monitoring by light traps. Significant long-term trends and cycles exhibited by light trap samples over the whole of Great Britain for the last 17 years have now been analysed as a background to studying more local environmental effects. A database of environmental information for all trap locations is currently being constructed from a large number of sources, so that temporal and spatial changes in moth diversity and species abundance can be related to known environmental effects. Particularly useful datasets on land use have been acquired or are being negotiated from MAFF, the Countryside Commission and the Nature Conservancy Council. Work is continuing on ascribing each light trap site to one of the Institute of Terrestrial Ecology's (ITE) 32 ecological land classes so that changes in moth population and community parameters can be related to ITE's survey information on changes in land use. The more detailed analysis of the long runs of data from Rothamsted farm is continuing, concentrating on the Barnfield trap for which well-documented information exists on changes in land use and agricultural practices. (Woiwod, A.M. Riley and Stewart)

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Pheromone studies on pea midge. Work aimed at producing a pheromone monitoring system for this pest has concentrated on the production of laboratory-reared insect material and the development of reliable laboratory bioassays.

Culture. After much preliminary work a continuous culture has been established on potted pea plants. Adults are kept over plants in cages for four days in an illuminated glasshouse, before infested plants are moved into a constant environment room at 25°C and *c.* 70% R.H. A single generation is completed in four weeks, the normal facultative larval diapause being eliminated by keeping all stages in a 18 : 6 (L/D) light regime. High larval mortality at the feeding stage has hampered mass rearing of this insect, but the number and quality of those produced are adequate for electrophysiological bioassays. Unusually, those used for such bioassays have been of a much higher quality than insects reared from field-collected larvae.

Laboratory bioassays. Electrophysiological recordings from whole antennae of males, or single circumfila loops, have been used to evaluate activity in crude pheromone extracts and gas chromatograph (GC) fractions. A special recording technique has been developed to overcome problems, apparently associated with the unusual morphology of the pheromone receptors. The receptor system is still not understood completely, but stable, sensitive preparations are now being obtained, thus enabling the use of coupled gas chromatography–electrophysiology to locate biologically active components of extracts.

A wind-tunnel bioassay has been developed, using a cylindrical observation chamber only 50 × 16 cm diam. and wind speeds of the order of 0.1 m s^{-1}.

Pheromone collection. Extracts with electrophysiological activity have been obtained by an air entrainment technique and by solvent extraction of excised ovipositors, but the most active extracts are those from solvent washes of glass vessels in which adult females have been kept.

Extracts from washed glassware have been fractionated by preparative GC and active fractions obtained but the recovery was poor. However, column-chromatographic clean-up procedures are now being used to produce electrophysiologically-active fractions which are being examined by coupled gas chromatography–electrophysiology. (Wall and Simpkins with Blight and Wadhams, Insecticides and Fungicides)

Radar studies. The Rothamsted Insect Survey Radar (RISR) was field tested for resistance to weathering and reliability of the associated electronic equipment. The unit has been working throughout the summer with occasional interruptions due to various tests and improvements to the system, but with no major breakdowns. Daily counts (accumulated over half-hourly periods) have been collected for a few days at a time at four height bands, and diurnal patterns of counts within each height band have been observed. An automatic transmission protocol has been implemented, and the computer in the field is sending the daily counts every morning via a telephone line and modem to a receiving computer in the laboratory. Progress has been made on the effect of 'noise' on the estimates of the target's parameters and the programme has been extended to include an evaluation of a mass range for each target.

Insect scattering properties have been based on the electromagnetic scattering properties of spheroids and ellipsoids which approximate to insects' shapes. The refinement of the approximations used will depend on further estimates of the information content of the real radar signal, which will ultimately decide how detailed a taxonomic key can be expected from this sort of equipment. The system has further proved to have the potential for a useful monitoring device, best exploited as part of a network of stations which would enhance the

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Insect Survey's capabilities to provide current information on the airborne insect pest situation in the UK. (Cuminetti, Prior; Dr J.Riley, Overseas Development Natural Resources Institute)

Biological control of pests

The importance of natural enemies of nematodes and aphids in the regulation of pest populations has been demonstrated but little is known about the factors that affect such control. Work has continued on the development of microbial parasites of nematodes and aphids as biological control agents that might be applied to crops.

Biological control of nematodes with *Pasteuria penetrans*. The obligate bacterial parasite of nematodes, *P. penetrans*, has potential as a biological control agent. The parasite has been recorded in many countries and on a number of plant-parasitic nematodes with individual populations of the organism exhibiting different host ranges.

Work has begun on collecting different populations of the parasite and determining their host ranges and pathogenicity to a number of root knot, cyst and free-living nematodes. The Department now has a total of 16 populations of *P. penetrans* all isolated from species of *Meloidogyne* of which 12 are regularly cultured *in vivo*. A standardized test has been developed to compare differences in the affinity of the parasite for its host; all populations investigated adhere only to the cuticles of species of *Meloidogyne*. The number of *P. penetrans* spores adhering to second-stage juveniles of *M. incognita* affects invasion. Invasion of tomato roots by second-stage juveniles, carrying 15 or more spores, was 86% less at high juvenile densities (1000 or 3000 roots per system) than at low densities (500 roots per system). Further studies have shown that the density of the second generation can be reduced by 93% when juveniles bearing 1−15 spores were added to soil compared to those without spores. Results so far indicate that there are differences in the aggressiveness between populations of *P. penetrans*. When juveniles bearing the same number of spores from two different *P. penetrans* populations were added around the roots of tomatoes there were differences in the number of females that subsequently became infected. (Davies, Flynn and Kerry)

Control of cyst nematodes by fungi. Control of the beet cyst nematode by naturally occurring fungal parasites is unlikely to reduce the populations below their economic thresholds. Hence, this natural control either needs to be integrated with other control measures or enhanced by adding artificially cultured fungi at levels that give acceptable control. Integration with low rates of nematicides and crop rotations are currently being tested in two long-term field trials at Woburn and Broom's Barn. Since the nematode undergoes a hatch in the Autumn for a third generation which never has time to develop, the effect of harvest date on the final population of the nematode has also been tested in a small field trial. Replicate plots were harvested on three dates (7 October, 12 November and 17 December), and soil samples were collected from all plots after the final harvest date. Although there was no significant difference in numbers of cysts between harvests, there was a significant reduction in the numbers of eggs from October to December (25 to 9 eggs g^{-1} respectively). Although harvest dates are more likely to be determined by factors such as weather conditions, this information could be of use when there are several fields to be harvested, in which case it would be beneficial to leave the more heavily infested fields until last to reduce the nematode population.

Work on fungal parasites of the potato cyst nematode began by examining three soils that had long histories of the nematode. Up to 50% of female nematodes developing on potato roots in these soils became infected. Fungal species isolated included *Verticillium*

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chlamydosporium, *Cylindrocarpon destructans* and *Fusarium* spp., all of which are known to be involved in the natural decline of cereal and beet cyst nematode populations. (Crump)

Biological control of aphids. Work on the development of the entomogenous fungus *Erynia neoaphidis* for aphid control has concerned firstly the development of an assay to test the fungus against aphids in a controlled environment on a scale intermediate between field and laboratory, and secondly the provision of genetically stable strains through isolations from solitary spores (monoconidial isolates).

In recent years natural populations of *Aphis fabae* on spring beans have been small, and naturally occurring aphid-specific predators prevented attempts to establish populations artificially by aphid release. As an alternative, aphid populations suitable for control testing were established in constant environment rooms but the rapid air circulation needed to ensure a uniform temperature in such facilities, precluded provision of the moisture conditions necessary for fungal transmission. Suitable conditions were, however, provided in a glasshouse where infested plants were periodically enclosed in a polythene tent within which the air was artificially humidified. Provisional results indicate establishment of the disease but the initial infection level four days after application of triturated cadavers of aphids killed by fungus was low (< 5%) and similar to that which occurs following application in the field. Surprisingly, spread of the infection during the subsequent two weeks was as good (> 20%) whether the air was humidified for 12h every three days or for 12h daily. Use of the humidifier without the polythene enclosure was insufficient to ensure fungal transmission. Current work is concentrated on application techniques that provide higher initial levels of infection.

Axenic cultures of Entomophthorales derived from aphids collected from the field decline in infectivity with successive subculturing. However, monoconidial isolates, especially of species with uninucleate spores like *E. neoaphidis*, may well be more stable. On nutrient media, conidia of *E. neoaphidis* will only germinate in the presence of numerous other conidia and it is impossible to isolate individual colonies originating from a solitary conidium. However, single conidia will germinate on the host and cause infection. Accordingly, to provide monoconidial isolates, pea aphids, *Acyrthosiphon pisum*, which are very susceptible to the fungus, were each inoculated with a solitary conidium transferred from a glass surface at the tip of a human hair. About 10% of the aphids so inoculated became infected and axenic cultures were readily established from the numerous conidia produced after the aphid died. Current work should determine whether such cultures will exhibit genetic uniformity and retain infectivity with successive subculturing. (Wilding and Sherlock)

New strategies for pest control

New methods for control frequently result from detailed studies of a pest's biology which may reveal vulnerable stages in its development which can be exploited. Also, those methods considered more acceptable environmentally, such as the use of natural enemies, crop rotation or resistant cultivars, tend to be specific to species or races of pests which are often difficult to distinguish morphologically. Research on the use of molecular and biochemical techniques for the identification of nematodes may have wide application in the development of simple and rapid tests for the diagnosis of some pest problems.

Observations on the response of the oesophageal glands of *G. rostochiensis* to hatch stimulation. Using video-enhanced contrast microscopy, the timing and nature of the response of the oesophageal glands of unhatched *G. rostochiensis* to hydration and hatch stimulation was studied. Secretory granule accumulation in subventral glands was solely a response to

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hydration of previously dried cyst contents. Accumulation of granules and increase in size of the gland cell nucleolus both occurred in dorsal glands in response to hydration and were further significantly affected by exposure to potato root diffusate (PRD). No secretory material was observed in the oesophagus or intestine and it is concluded that the oesophageal glands are not involved in the process of eclosion and the limited response to PRD was part of the preparation of the juveniles for a feeding phase after hatching. Thus, to cause hatching in the absence of host plants, it will probably be unnecessary to induce enzyme action. (Perry with Prof. U. Wyss and Dr U. Zunke, University of Kiel, FRG)

Molecular and biochemical methods of identification of potato cyst nematodes. DNA restriction fragments derived from repetitive DNA have been shown to differentiate populations of *G. rostochiensis* and *G. pallida* (Burrows and Boffey, *Revue de Nématologie* (1986) **9**, 199-200).

In order to exploit DNA sequences that occur much less frequently they must be cloned. A genomic library has been prepared from *G. pallida* using the plasmid vector/host system pUC-9/*E. coli* JM83. Recombinants were selected and screened using *in-situ* hybridization techniques for *G. pallida* specific DNA sequences. Two such 'probes' have been isolated and are presently being screened against a wider range of *G. rostochiensis* and *G. pallida* populations. (Burrows)

Close collaboration with the Zoology Department, Coimbra University, Portugal, is providing valuable information on the European distribution of *G. rostochiensis* and *G. pallida*. In an extensive survey in Portugal, isoelectric focusing techniques (IEF) were used to identify field populations of *G. rostochiensis* and *G. pallida*. Subsequent to the first record of *G. pallida* in Portugal (*Rothamsted Report for 1986*, 112) further populations of *G. pallida* have been found, one of which produces an atypical IEF protein pattern. More cysts are being collected so that the morphology may be studied and the IEF repeated at Rothamsted to determine if the unusual protein pattern is consistent.This may be an important exception to what is now a well established identification technique. (Burrows with Prof. Maria Susana de Almeida Santos, University of Coimbra)

Biochemical identification of root knot nematodes. Work continued to develop electrophoretic methods for root-knot nematode (*Meloidogyne* spp.) characterization. Ultrathin gel isoelectric focusing in conjunction with high voltage gradient separation of soluble proteins has been found to be the most economic and reproducible method of nematode identification. This technique has been used to screen the Departmental collection of *Meloidogyne* species and host-races. Total protein stains (Coomassie Brilliant Blue and silver stain) have revealed species-specific but not host-race-specific protein differences. High resolution non-specific esterase stains have also been used to characterize *M. incognita, M. arenaria, M. javanica*, and *M. hapla*. Much less expensive and simpler mini-gel isoelectric focusing apparatus has proved as efficient as more conventional systems in separating the major species of root-knot nematode. (Robinson with Dr S.B. Jepson, University of Southampton)

Cuticular proteins of root-knot nematodes. Surface proteins from intact second-stage and adult females of *M. incognita* Race 1 were partially characterized through radio-iodination. Detergent and β-mercaptoethanol extractions of labelled cuticles were separated by SDS-polyacrylamide gel electrophoresis. Juvenile and adult stages were distinct in both extracts and each was characterized by a limited number of major bands. Differences between the stages suggest that the basic structure of the cuticle changes during moulting. Further analysis

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of the cuticular components may provide information which will enable the development of new approaches to detect and control root-knot nematodes. (Robinson with Dr R.M.E. Parkhouse, National Institute for Medical Research, Mill Hill, London)

Slug control. The effectiveness of the novel slug poison previously reported has been confirmed in field trials (*Rothamsted Report for 1985*, 100). It was most effective as a stomach-action poison incorporated into edible bait pellets, as a 5% a.i. formulation. The toxic mechanism is unknown as yet, but is apparently irreversible as fewer than 2% of slugs found immobilized on the soil surface in trials subsequently recovered. Structure/activity tests with related chemical analogues are continuing. Lack of persistence has prevented exploitation of recently discovered antifeedants as cereal seed dressings and foliar sprays; attempts to overcome this by appropriate formulation are in progress in collaboration with several industrial R & D departments. (Henderson, Parker and Turner, with Coward and Pickett, Insecticides and Fungicides, and Dr J.I. Bullock, University of Surrey)

Staff and visitors

Regrettably, the posts of a further two members of staff, Diana Parrott and Janice Payne, were declared redundant. Fortunately, Janice has been redeployed within the Department, but sadly, Diana left in March after 24 years' service; her considerable contributions to research on potato cyst nematodes have been greatly missed. The Department is indebted to D.J. Hooper who, following A.R. Stone's untimely death, has been 'acting Head' of Nematology until the formation of the new Department.

We congratulate Ingrid Williams, D.H. Crump and A.M. Riley on their promotions. Joyce Johnson, Phoebe Smith and L.C. Haynes retired and P.L. Sherlock, Veronica French, J. Hargreaves, S. Prior, Deborah Game, I.A. Kirkwood, C.G. Peters, D.A.C. Marshall, G.J.A. Radcliffe and Jane Barba resigned. It is a pleasure to thank them for their varied contributions to the life and research of the Department. Ann Hunter, G.H. Turner, Elspeth Bartlet, Lynda Alderson, Marie Rogers, Maria Leijdens, K.G. Brasier and S.I. Richardson were appointed. J.J. Feil and P.P.J. Haydock were registered for Ph.D. projects to study host effects on the development of *D. dipsaci* with the University of Leeds, and tolerance to potato cyst nematodes with Luton College of Higher Education respectively. Karen Saunders and S.A. Bowen completed their studies for a Ph.D.

Several members of the Department made contributions at the following conferences abroad: International Conference on Africanised Honey Bees and Bee Mites, Columbus, USA (B. Ball), 7th International Entomophagous Insects Workshop, University of Maryland, USA (W. Powell), 7th International Rapeseed Congress, Poznan, Poland (I.H. Williams), Meeting of the Society of Nematologists, Hawaii, USA (K. Evans), OILB/EEC, Euraphid-Dynamics and Identification of Aphids training course, Montpellier, France (G.M. Tatchell, I.P. Woiwod), CIMMYT BYDV Workshop, Udine, Italy (N.Carter, D.Morgan), Workshop on Interactions between Soil-inhabiting Invertebrates and Micro-organisms in Relation to Plant Growth, Columbus, USA, (B.R. Kerry), International Symposium on Crop Protection, Gent, Belgium (N. Wilding), Annual meeting of the Entomological Society of America, special symposium on 'Practical Applications of Insect Pheromones', Boston, USA (C. Wall). Departmental staff also contributed to various meetings within the UK and P.R. Burrows, D.J. Hooper and B.R. Kerry gave lectures on the Commonwealth Institute of Parasitology course in Nematology.

B.V. Ball spent three weeks at the Tierhygienisches Institut, Freiburg, W.Germany, working on bee diseases as part of a collaborative EEC funded project. E.A. Stafford continued her

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field studies on heavy metal uptake funded by the United States Army Corps of Engineers, and spent eight months working at the Waterways Experiment Station, Vicksburg, USA. G.M. Tatchell, I.P. Woiwod and E.D.M. Macaulay visited INRA laboratories in Le Rheu, France, to discuss suction trapping for aphids as part of the INRA/AFRC co-operation scheme. N. Wilding visited the Station de Zoologie Appliquée de l'Etat, Gembloux, Belgium. D.J. Hooper was invited to advise on nematode taxonomy at the Norwegian Plant Protection Institute, Ås, and Swedish University of Agricultural Science centres at Uppsala and Alnarp. He also visited nematology laboratories in Beijing, Nanjing and Shanghai, China, under the auspices of the British Council's Academic Links with China Scheme. K. Evans visited the Universities of Coimbra and Tras-os-Montes, in Portugal to advise on potato cyst nematode survey methods and to set up experiments to determine the distribution of pathotypes.

P.R. Burrows and M.P. Robinson were also invited to the University of Coimbra to demonstrate biochemical methods for nematode identification. A.G. Whitehead visited laboratories in Rennes, Lusignan, Paris, Boigneville and Antibes, France, Assen and Wageningen, The Netherlands, and Nyon, Switzerland to discuss nematode problems on break crops and integrated control.

The Department was pleased to receive many short term visitors who came for training and to exchange information. Longer term visitors were:
Wu Ju-wen, Beijing Academy of Agricultural & Forestry Sciences, China, (biological control of insects), Lin Maosong, Nanjing Agricultural University, China, (nematode taxonomy), Dr. K. Richards, Lethbridge Research Station, Canada, (pollination by honey bees). Marie Winder, Rowena George, D. Wood, I.J. Wyatt, and Julia Hanmer were employed as field scouts to sample cereal aphids.

Support for our research programme from the EEC, Home Office, British Technology Group, Overseas Development Administration, Nuffield Foundation, Ciba Geigy, US Army, Agricultural Genetics Company, Perry Foundation, ICI and Shell is acknowledged.

INSECTICIDES AND FUNGICIDES DEPARTMENT

The Department continues to concentrate on research towards improved effectiveness of chemical crop protection by avoiding or containing resistance in agricultural pests, and also reducing still further adverse effects on the environment. Recognition of success in these endeavours has enabled further acquisition of temporary commercial funding, but this funding has only been accepted where an appropriate balance could be maintained with the core-funded strategic work within the five main-line projects discussed below. However, some further cuts in core-funding have occurred, and will result in the loss of a prestigious and extremely valuable post in the Department unless permanent funding can be obtained for new work utilizing the particular expertise involved. Nonetheless, we are delighted to have been granted a new chemistry post, to complement a new post in the Biochemistry Department, for collaborative work on the biosynthesis and genetics of semiochemicals particularly of the drimane antifeedants produced by water-pepper, *Polygonum hydropiper*.

Dr. Roman M. Sawicki, who has made an enormous contribution to the long-term study of insecticide resistance both personally and in his leadership of the Resistance Group in the Department, was elected a Fellow of the Royal Society and we are pleased to join the many who congratulate him in this recognition of the scientific standing of his work.

The synthesis and identification of new biologically active compounds have led to further discoveries about the interaction between chemical agents and various pests and about the biochemical targets involved. The most dramatic developments reported are the proof that gene amplification is responsible for the increased synthesis of the esterase that confers

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insecticide resistance in the aphid *Myzus persicae*, and the further elucidation of the genetic changes that occur when aphids lose resistance.

Over the next year we look forward to initiating new lines of work, involving further combinations of chemical and molecular biological approaches, and also in obtaining further outside funding for these studies.

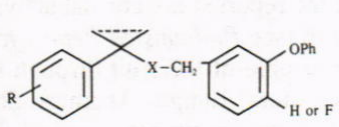
Relationship between molecular structure and insecticidal activity

As concern increases, both for the environment and about the rising number of species of insect pests resistant to insecticides, the need for more effective, yet easily degraded, control agents becomes more urgent. Our aims, in response to these problems, remain the discovery of new classes of insecticidal compound followed by the identification of the structural features essential for their activity. Opportunities to enhance either performance against resistant insects or selectivity against pests relative to their predators continue to be exploited as fully as possible.

Synthetic pyrethroids with a non-ester central group. Substantial changes in both the acid and alcohol components of pyrethroid esters can be made in order to produce insecticides with improved properties, and modifications to the central ester link are also possible (*Rothamsted Report for 1984*, 102) leading to compounds represented by formula (I). More detailed investigations in this series, involving the synthesis and testing of over 70 compounds, have shown that changes in insecticidal activity consequent on changes in structure may or may not depend on the other groups present in the molecule, i.e. that structure-activity effects may in some circumstances be significantly non-additive, as has been observed previously in the ester series.

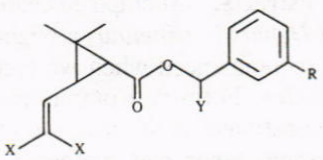
For instance, the change in formula (I) from dimethylmethine (-CMe_2-; '– – – –' = no bond) to cyclopropyl ('– – – –' = a bond) studied over a range of compounds enhances activity more than threefold (on average) when X = -CH=CH- or -CH_2CH_2-, but has little effect when X = -CH_2O-.

On the other hand, the enhancement of activity on changing the 4-substituent from H to F (*Rothamsted Report for 1985*, 104) is maintained in the wider series of results now available, and no significant dependence on structural features elsewhere in the molecule was detected. Similarly, over a wider range of aromatic substituents (R in formula (I)), no dependence of the result of substitution on the groups present elsewhere could be discerned. In this latter study, the range of R groups introduced was widened to include greater extremes of polarity and electronic properties; the insecticidal activities of these compounds established constraints on structural variation which are valuable in defining areas for further examination.



(I) ----- = bond or no bond

X = $-CH\overset{E}{=}CH-$, $-(CH_2)_2-$ or $-CH_2O-$



(II)

Is a double bond in the alcoholic side-chain of pyrethroids essential for activity? Highly active pyrethroids, for instance benzyl esters of cyclopropane acids such as (II), all have R groups containing some form of unsaturation. The unsaturation may be aromatic

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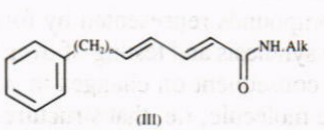
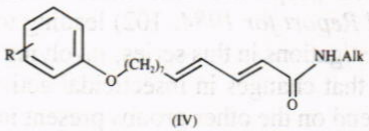
(R = -OPh, $-CH_2Ph$), olefinic (R = $-CH_2CH=CH_2$) or imine-like (R = -CH=NOR or $-CH_2CH=NOR$). Corresponding compounds lacking the unsaturation are generally much less active.

The cyclopropyl group can be considered as containing latent unsaturation, and so can be used as a test of the function of unsaturation in binding to the lethal site.Several series of compounds represented by II (X=CH_3 or Br, Y=H or CN) were examined. In each case, the known allyl compound (R = $-CH_2CH=CH_2$) was hydrogenated to give the saturated compound (R = $-(CH_2)_2CH_3$) or subjected to Simmons-Smith reaction to give the methylene addition product (R = $-CH_2\overline{CH.CH_2}CH_2$). Bioassays showed that the cyclopropyl compounds were intermediate in insecticidal activity between the allyl and propyl. It therefore appears that π electrons, available either directly or consequent on ring opening, are an important feature for high activity.

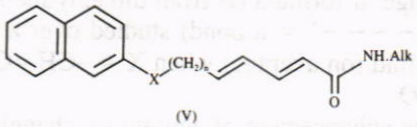
***N*-Alkylamides.** Our earlier work (*Rothamsted Report for 1984*, 103) showed that in the series represented by structure (III) a peak of activity was observed around n = 8, as well as at n = 1, an observation consistent with the activity found by Miyakado *et al* in compounds such as (IV) (*Journal of Pesticide Science* (1985), **10**, 25–30).



(III)



(IV)



(V)

We have now synthesized and tested three series of analogous naphthyl-terminated long-chain compounds (V) with X = -O-, -CH=CH-, or a direct bond. The requirements for chain length are much more specific than in the phenyl-terminated case with significant activity being observed for one member only of each series, that in which the number of chain-forming atoms (i.e. in the composite unit $-X-(CH_2)_n-$) was eight. Clearly chain length is a dominant feature in determining insecticidal activity, whereas the nature of X is of less importance.

***Dennettia* extracts.** Attempts to characterize the reported insecticidal activity of extracts of the fruit of *Dennettia tripetala*, a Nigerian shrub (see *Rothamsted Report for 1982, Part 1*, 122) were recommenced when we received a sample of the fruit through the kind help of Dr J.C. Okafor, Forestry Commission Headquarters, Enugu, Anambra State, Nigeria.

The major constituent in the material extracted by successive treatment of the macerated fruit with hexane, ether and methanol was confirmed by nuclear magnetic resonance spectroscopy (comparison of ^1H and ^{13}C spectra with those of an authentic synthetic sample) to be 2-phenylnitroethane, as previously reported. However, none of the extracts showed significant insecticidal activity against any of our test species (*Musca domestica, Phaedon cochleariae* and *Plutella xylostella*). We conclude that *Dennettia* does not contain significant quantities of any compound that might serve as a suitable lead for a structure/activity study of the type undertaken with natural pyrethrins and the *N*-alkylamides.

(Chemical work: Janes, Khambay, Ifill; Biological work: Farnham, Orgill, Timby)

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Resistance to insecticides

Advances in biochemical monitoring and studies on selection for resistance in age-structured populations of houseflies and whitefly in the laboratory, and to peach-potato aphid in the field, are contributing towards understanding selection processes for insecticide resistance. These data are used in computer simulations to identify factors that accelerate and retard resistance, an important step in developing effective resistance management strategies.

Further biochemical investigations have demonstrated conclusively that resistance in *M. persicae* depends on the amplification of the E4 gene.

Evaluation of insecticidal mixtures for managing resistance. Current theoretical studies predict that resistance is best delayed by using mixtures of insecticides when controlling populations.

To test this prediction we have started a major multidisciplinary project in which housefly populations, with fully characterized resistance mechanisms, are subjected in the laboratory to regimes of insecticides of several chemical classes applied singly or in mixtures under realistic exposure conditions in large 'population' cages. Progress relevant to this aim has been in several areas.

1. Starting from multi-resistant strains of houseflies, several individual resistance mechanisms (*kdr* and its variants; Dld_4; $E_{0.39}$; $AChE_R$) have been isolated. They represent a wide range of resistance types to different classes of insecticides (organochlorines, cyclodienes, organophosphates, pyrethroids). Each of these mechanisms, which differ in their phenotypic expression and interaction with other resistance genes, is being inbred into the same genetic background to eliminate extraneous genetic effects.
2. Biochemical and bioassay techniques to detect and monitor these mechanisms both in individual insects and in selected populations have been developed and appraised. A technique described previously (*Rothamsted Report for 1983*, 104) for characterizing the acetylcholinesterase (AChE) of individual insects has been adapted for 96-well microplates. Different enzyme forms are now identified in homozygous and heterozygous condition with the same precision as in the original method, but much more rapidly and with greater sensitivity. The effects of several inhibitors can thus be measured giving an 'insensitivity profile' for each insect; this enables us to isolate housefly strains, homozygous for different forms of AChE, from mixed populations.
3. The effect of physical and chemical properties of residues of insecticide mixtures on population size and selection rate in large cages has been investigated. An evaluation of permethrin and trichlorfon has shown that differences in factors such as repellency (by permethrin only) and loss of activity of the residues substantially complicate the formulation of mathematically ideal mixtures, the components of which are assumed to contribute equally in controlling the pest.

Work is in hand to determine how far the addition of trichlorfon, which is equally effective against flies with and without *kdr*, protects pyrethroids from this resistance mechanism.

4. Data from population-cage experiments are used for predictive computer modelling to provide the theoretical framework for appraising the longer term effect of mixtures on selecting for resistance, and for optimizing this approach to managing resistance.

The model developed to describe the effect of pyrethroid residues on age-structured populations containing *kdr* (*Rothamsted Report for 1986*, 97) has been substantially modified using additional data on the efficacy and persistence of pyrethroids and organophosphates, and is being extended to handle the effects of selection by mixtures of two independent resistance mechanisms in the same population.

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Automatic selection for resistance. In the standard procedure for selecting resistant houseflies, adults, sexed on emergence to prevent mating, are treated individually with a discriminating dose of insecticide. To save time and labour on the routine selections of thousands of insects, we have devised a technique in which newly emerged flies select themselves on emergence.

Clean pupae are put in a dark cylindrical container lined with aluminium foil coated with a residue of the selecting insecticide. From these pupae, newly emerged flies, attracted by the light at the open end of the container, crawl over the treated surface, pick up the discriminating dose of poison and are thus automatically selected for resistance. By choosing the strength of the residue of permethrin we discriminate in these selections between flies with *super kdr* or *kdr*, the heterozygotes of either strain, and the susceptible flies.

Genetics of sex-determination in houseflies. The increasing occurrence of autosomal sex determinants in housefly populations, which probably reflects the activity of transposable elements, continues to complicate the genetic analysis of insecticide-resistant strains. The discovery in two strains from South Africa of a male determinant on autosome III (MIII) and an unlocalized female determinant (F) that counters the effect of MIII in female houseflies considerably extends the known distribution of autosomal sex determinants in houseflies worldwide.

Resistance in the tobacco whitefly, *Bemisia tabaci*. We have developed techniques for monitoring age-structured populations of the tobacco whitefly in the laboratory, and for testing the effects of insecticide treatments in ways that overcome the shortcomings of conventional laboratory bioassays and field trials. Tests with existing and new compounds have proved successful, and the diagnosis of resistance mechanisms in individual insects of the field strain from Sudan is yielding to biochemical analysis.

Selection for resistant *M. persicae* in field cages. Residues of pirimicarb, or deltamethrin/heptenophos, sprayed on potatoes in field cages reduced artificial infestations of susceptible(S) and resistant *M. persicae* introduced up to 14 days after treatment. The deltamethrin/heptenophos formulation controlled aphids (particularly nymphs) better than pirimicarb, but selected more strongly for very resistant (R_2) aphids due to the combination of its greater persistence and the higher resistance to pyrethroid than carbamate insecticides. Surprisingly, S aphids survived the pyrethroid treatment better than R_1 aphids, suggesting a difference in behavioural response to the pyrethroid. (with Clark, Statistics)

Resistance of *M. persicae* on unsprayed field crops. Aphids were collected from brassicae in October-November 1986 and from potatoes, sugar beet and brassicae during June-August 1987, and analysed by the E4 immunoassay (*Rothamsted Report for 1985*, 109) to determine their resistance. Throughout the country moderately resistant aphids (R_1) were most common, but the increased incidence of very resistant aphids (R_2) (even on untreated crops) remained high. At one extreme, in Lincolnshire and East Anglia, S aphids were rare (12%) and very resistant insects accounted for 20% of the aphids sampled; at the other, in Shropshire, Staffordshire and Hampshire, S aphids predominated (40-50%) and R_2 aphids were rare (<10%). (with Loxdale, Entomology and Nematology)

Selection for resistant *M. persicae* by spraying field crops. See this year's reports for Broom's Barn and Entomology and Nematology Department. (with Dewar and Harrington)

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Loss and reselection of resistance in *M. persicae* clones. Some of the most resistant (R_3) aphid clones, which have an A1,3 translocation, spontaneously lose resistance and, concomitantly, their high levels of E4. In ten R_3 clones from the field, the pattern of loss, monitored by the E4 immunoassay, varied markedly between clones; when catastrophic loss occurred, a high proportion of susceptible individuals was produced within one generation without the formation of stable intermediates. Reselection of resistance by omethoate in reverted susceptible sub-clones acted on existing intraclonal variation in E4 levels which was not apparent in susceptible clones of normal karyotype. These changes in E4 production were not associated with a change in karyotype. The ability of revertant aphids, unlike normal susceptible insects, to respond rapidly to selection clearly has important implications for understanding the development of resistance in the field. (with White, Statistics)

Genetic basis of insecticide resistance in *M. persicae*. Hybrid-selection techniques confirmed that two of the clones in a cDNA library prepared from very resistant *M. persicae* (*Rothamsted Report for 1986*, 99) correspond to the gene for E4, the enzyme causing insecticide resistance. Using this cDNA to study differences in E4-related nucleic acids between susceptible and resistant aphids established conclusively that the increased amount of esterase in resistant aphids results from gene amplification, i.e. multiple copies of the structural gene. This appears to be associated with chromosome translocation since restriction analysis of DNA from resistant aphids, either of normal karyotype (e.g. R_1) or with the translocation (R_2,R_3), showed differences in the arrangement of amplified esterase genes. Aphid clones that had spontaneously lost resistance (see above) had also lost their elevated E4 mRNA but retained their amplified DNA, demonstrating that transcriptional control of the amplified genes is a further regulatory mechanism responsible for resistance. Using the E4 probe to analyse the DNA of individual aphids from wild populations thus serves as a valuable complement to the E4 immunoassay, since it readily distinguishes susceptible and reverted individuals. (with B.G. Forde, Biochemistry)

(Sawicki; Byrne, Denholm, Devonshire, Dunckley, Farnham, ffrench-Constant, Field, Hackett, Moores, Rowland, Stribley, White, Williams)

Semiochemicals

Examples continue to accumulate of semiochemicals (behaviour-controlling chemicals) contributing to reduced insecticide usage either by directly replacing insecticides or by use in integrated pest management programmes. Our sophisticated analytical equipment and unique expertise in its use, particularly in the analysis by gas chromatography coupled with single sensory-cell recordings have allowed further, relatively rapid, characterization of semiochemicals. Synthesis of these compounds and analogues and testing in bioassays relevant to eventual field trials has enabled this group, in collaboration with the Systems for Novel Crop Protection Agents Group, to identify and tackle strategic problems involved in using semiochemicals in commercial agriculture.

Antifeedants. To extend the successful demonstration that the plant-derived drimane antifeedant (−)-polygodial (VI) can be used to control aphid-borne plant virus disease in the field, a series of 20 related synthetic and natural drimanes has been investigated. Only (−)-warburganal (VIII) (from the East African tree *Warburgia ugandensis*) showed activity comparable with that of (−)-polygodial. The synthetic compounds dimethyl polygodioate (VII) and methyl 9a-hydroxydrimenoate (IX), known to have levels of activity against lepidopteran larvae similar to those of the two natural products, were almost inactive against

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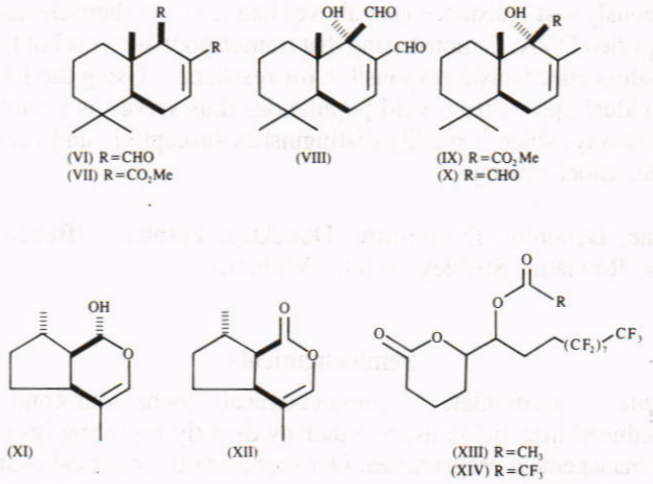
aphids. In order to investigate the possibility that for aphid activity the aldehydic function is necessary, the 9a-hydroxydrimenal (X) was tested but, although this compound was even more active than (−)-polygodial against diamondback moth, it proved inactive against aphids. (with Prof. S.V. Ley, Imperial College of Science and Technology, London, and Dr J-Y. Lallemand, Ecole Polytechnique, Paris, France)

New HPLC analysis methods mentioned in earlier Reports, e.g. *Rothamsted Report for 1985*, 106, have now enabled comparative assessment of the biological activity of (−)-polygodial and its unnatural (+)-enantiomer. Contrary to earlier indications, the antifeedant activity and phytotoxicity of the isomers are similar. Phytotoxicity occurs at levels higher than those required for antifeedant activity. Furthermore, studies with aphids feeding on artificial diets have demonstrated that antifeedant effects are independent of phytotoxic effects in the plant. The hot-tasting sensation, assessed by a human taste panel, was observed for both the (−)- and (+)- isomers. (with Prof. K. Mori, University of Tokyo, and Prof. Y. Asakawa, Tokushima Bunri University, Japan). The enantiomers of polygodial reacted at different rates with enantiomers of 1-phenylethylamine: from the partial reaction of (±)-polygodial with (+)-(*R*)- and (−)-(*S*)-1-phenylethylamine, the recovered polygodial gave specific rotations ($[\alpha]_D^{22}$) of −10° and +9° respectively. Although these rotations are small compared with that of pure natural (−)-polygodial, ($[\alpha]_D^{24} = -131°$), they clearly indicate stereo- differentiation and make the similar biological activity of the polygodial enantiomers more surprising.



Aphid sex pheromone. The identification of the sex pheromone of the aphid *Megoura viciae* (*Rothamsted Report for 1986*, 100) did not include the absolute stereochemistry at C-1 of the lactol. This has now been established as (*R*) (compound XI) by X-ray crystallography on the 3,5-dinitrobenzoate. (with Dr D. Williams, Imperial College of Science and Technology, London). Compound XI and the lactone (XII) have now also been identified in the black bean aphid, *Aphis fabae*, and the pea aphid, *Acyrthosiphon pisum*, with the lactone predominating in the former and the lactol in the latter. A synthetic route to these compounds has been developed from limonene epoxide, thus making material available for further biological assessment.

Mosquitoes. The oviposition-attractant pheromone, prepared by the route described earlier (*Rothamsted Report for 1985*, 107), has been used successfully in field trials in Kenya to

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attract mosquitoes to lay eggs, the larvae from which were then destroyed by an insect growth regulator incorporated with the pheromone formulation. The need for analogues more volatile than the parent pheromone has led to the preparation of the heptadecafluoro compound (XIII) which shows high attractant activity. Further fluorination to compound (XIV) removed activity, possibly through hydrolytic instability. The retention of activity after this high level of fluorination in the side chain suggests that a spatial interaction with the pheromone receptor in the mosquito is involved rather than the expected lipophilic interaction. (with Dr B.R. Laurence and Dr M.M. Pile, London School of Hygiene and Tropical Medicine, and Dr W.A. Otieno and Mr T.O. Onyango, International Centre of Insect Physiology and Ecology, Nairobi, Kenya, supported by the British Technology Group (BTG))

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

Systems for application of novel crop protection agents

The matching of formulation, application method and timing to the nature of the chemical and its desired effect on the pest requires individual optimization for each pest problem. Our efforts in this area on potato diseases have been completed; other work reported this year concerns the use of alarm pheromone against aphid pests.

Development of an electrostatic sprayer for potato tubers. The sprayer system described earlier (*Rothamsted Report for 1984*, 111) has been developed further, and tested for efficiency of application to seed potato tubers judged by decreased levels of *Polyscytalum pustulans* (skin spot). The charged rotary atomiser gave over twice the deposit of the fungicide (imazalil) compared with a hydraulic nozzle spray, and levels of skin spot in the subsequent crop, both at harvest (10% vs. 26%; no treatment 49%) and after further storage (11% vs. 31%; no treatment 50%), were significantly lower.

Patent rights for the system have been assigned to BTG who have provided financial support to produce prototype sprayers for evaluation by the Potato Marketing Board. The system is now at the stage where commercial development under licence is appropriate.

Formulations of aphid alarm pheromone. Good results have been obtained with hexane formulations of synthetic aphid alarm pheromone, (*E*)-β-farnesene, in the field (*Rothamsted Report for 1986*, 101), but established farm practice requires formulations that can be dispensed by hydraulic sprayers.

The effect of formulation solvent on the action of the aphid alarm pheromone was studied by applying single drops of pheromone close to colonies of aphids. For solvents of one compound type (e.g. the alkanes), there is a direct relationship between solvent vapour pressure and the effectiveness of the solution in producing alarm response. Aphid response was generally poor when solvents with vapour pressure <70 Pa were used.

Experimental aqueous formulations designed to liberate farnesene quickly have been tested in the new field-simulation facility, and the most active were then tested in the field in combination with a contact pesticide. Although some promising results have been obtained with aqueous formulations, none has yet achieved the reliability of farnesene in hexane.

(Griffiths; Pye, Smart, Woodcock)

Pesticide distribution in plants and soils

Understanding the movement and persistence of pesticides following application to soil or plants is a prime consideration when searching for new, safer products, since the compound

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needs to reach its site of action and yet not move to where its presence is undesirable e.g. ground water. One of our particular interests is that a number of pest and disease problems are not controlled efficiently by currently available pesticides and could in theory be better controlled by phloem-mobile insecticides and fungicides if these were available.

Chlorsulfuron damage to sugar beet. Previous investigations (*Rothamsted Report for 1986,* 102) of the cause of damage to sugar beet grown in 1986, following application of the sulphonylurea herbicide chlorsulfuron in 1984, indicated that most incidents occurred on poorly drained soils or at low lying sites with a water table close to the surface. Damage to sugar beet grown in 1987 following application of the herbicide to cereals in 1985 followed this same pattern: 55 cases occurred on pelosols and stagnogleys which are impermeable, 60 cases on gleys which are intermittently waterlogged, and 16 on clay enriched argillic subsoils. In addition, 30 cases were on apparently free draining sites, but most of these were in the exceptionally alkaline sandy region south west of Bury St Edmunds; reduced degradation at high pH may therefore be an additional factor in determining persistence.

The consequences of applying mobile pesticides in autumn to soils where drainage is impeded or where the water-table is close to the surface need to be understood before a new pesticide is registered as a soil treatment.

Synthesis of volatile fungicides. Investigations have continued into the factors affecting the activity of soil-applied fungicides. Redistribution via the vapour phase is potentially important; it is known, for example, that the activity of lipophilic insecticides in soil is enhanced by increased volatility. However, most commercial fungicides have relatively low vapour pressures.

To investigate how changes in chemical structure affect vapour pressure and activity in soil, several analogues of penconazole (vapour pressure 210μPa), a triazole fungicide which inhibits ergosterol biosynthesis, have been synthesized. Simple changes in structure have led to compounds with vapour pressures up to 120 mPa but which, in simple agar tests, retain activity, albeit lower than that of penconazole, against the fungus, take-all (*Gaeumannomyces graminis* var. *tritici*). In further tests designed to measure activity following evaporation from glass and from soil (*Rothamsted Report for 1986*, 101), some of the new compounds were more active than penconazole, showing that their higher vapour pressure outweighs their lower intrinsic fungitoxicity. Further tests are required to compare the effectiveness in soil of these volatile compounds with that of other related fungicides such as PP969 whose redistribution is primarily via the soil water.

Phloem translocation. Many aspects of transport in phloem sieve tubes are poorly understood and even the basic mechanism is still a source of controversy. The most widely supported theory, the 'pressure-flow hypothesis' advocated by Münch, (Münch, E (1930) *Die Stoffbewegungen in der Pflanze.* Jena: Gustav Fischer) proposes that sucrose is loaded into leaf phloem by a specific carrier mechanism; water then diffuses into the phloem sieve tubes to equalize the osmotic pressure and, since the sieve tubes are essentially inelastic, the solution is displaced to regions where the osmotic pressure is lower due to the sucrose being utilized for plant growth.

The existence of specific carrier mechanisms has been proposed for other endogenous compounds, such as amino acids and plant hormones, and even for synthetic pesticides, though the conclusion from our earlier work (*Rothamsted Report for 1985*, 111) was that appropriate physico-chemical properties alone may well explain phloem transport of most of these compounds. We are now examining the phloem transport of several pairs of ^{14}C-labelled

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enantiomers, both natural and xenobiotic, using our test system based on *Ricinus communis*; pairs of enantiomers have the same properties of polarity, water solubility and ionization and so would be expected to be transported similarly if these properties were the sole determinant, whilst differences in transport between stereoisomers would indicate the involvement of carriers acting preferentially, as do enzymes, on one enantiomer.

Tests with 2-methoxy-2-phenylpropanoic acid, an analogue of the plant hormone phenylacetic acid, indicated no difference in the rate of appearance of the two enantiomers in stem phloem over the six hours following application (2 mM aqueous solution) by petiole injection, nor in the distribution of chemical in the plant 24 hours after application. Rather more surprisingly, no differences were observed in the appearance in stem phloem of D- and L-phenylalanine, even though the latter is an important protein amino acid. Further work is in progress, but these preliminary results do not provide evidence for specific carriers for these compounds.

The behaviour of other, achiral analogues of phenylacetic acid has also been examined. Varying the substituents in the phenyl ring gave compounds with a range of polarity whilst keeping a constant acid strength (pKa $\approx$ 4). Although the phenylacetic acids were in general too rapidly metabolized for comparisons of distribution patterns to be made, it was clear that compounds of intermediate lipophilicity were the best translocated in phloem, as previously observed for phenoxyacetic acids (pKa $\approx$ 3). Many plant hormones are weak acids of intermediate lipophilicity and could therefore undergo similar passive transport in phloem. It is possible that the movement of even these endogenous compounds is determined more by their availability for transport than by specific control within the transport process itself.

(Bromilow; Chamberlain, Evans, Nicholls, Tench, Williams)

Staff and visitors

Diana M. Johnson took early retirement after more than 25 years in the AFRS. M.C. Smith resigned from his temporary MAFF-contracted post and was replaced by Sangita J. Shah. M.M. Patel was appointed to a new permanent post to work on biosynthesis of antifeedants. A.J. Tench and R.H. Williams were appointed to new posts funded by ICI for approximately three years to study phloem translocation of pesticides, and Nicola P. Coward was appointed to an additional BTG-funded post to work on new molluscicides. In posts funded by BTG, T. Javed and Jacqueline Holden left at the end of their contracts, and Joanne Orgill and S.P. Kingham were appointed to new posts. Toni M. Fleming worked with A.L. Devonshire for two months on insecticide resistance, supported by a grant from four agrochemical companies. B.M. Venning and Rachel L. Dunckley resigned from their AFRC New Initiative posts, the latter transferring to the Biochemistry Department; neither was replaced.

R.M. Sawicki was elected a Fellow of the Royal Society. M.W. Rowland and R.H. ffrench-Constant were each awarded the degree of Ph.D. from the University of London.

Among visiting scientists welcomed to the Department was Dr. Liu Xun from the People's Republic of China, who was making a return visit for two months to establish a new collaborative programme on insect pheromones. Dr. Angel Guerrero made a brief visit to the Department.

Staff made several visits abroad. J.A. Pickett and L.J. Wadhams each visited Spain to conclude the programme of collaboration with the Instituto de Química Bio-orgánica, Barcelona, supported by a British Council grant. It is hoped that the project can be expanded, however, through an application for EEC funding. A.L. Devonshire visited the People's Republic of China, supported by a Royal Society grant, to lecture and discuss common research interests in insecticide resistance. R.M. Sawicki was invited to Australia by the Australian Agricultural

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and Veterinary Chemicals Association, with expenses paid by the Pyrethroid Evaluation Group, to discuss aspects of insecticide resistance management; he was also invited to give a paper at the Annual Meeting of the American Entomological Society in Boston. In addition, he organized the resistance session at the 11th International Crop Protection Conference held in the Philippines, and travelled on to Japan by invitation of the Sumitomo Chemical Company for discussions. J.A. Pickett visited two INRA establishments in France to discuss collaborative work and give a seminar; he also lectured at the International Euchem Conference in Angers, France. D.C. Griffiths presented a paper at the 14th International Symposium on Controlled Release of Bioactive Materials in Toronto, Canada, and P.H. Nicholls participated in the International Sulphonylureas in Soils Workshop in Wilmington, Delaware, organized by Du Pont; he also visited the Cyanamid laboratories in Princeton. R.H. Bromilow and P.H. Nicholls were invited to the Ciba-Geigy laboratories in Basle for discussions and to give presentations on their work.

Several staff attended the annual meeting of the International Society of Chemical Ecology in Hull at which J.A. Pickett presented a paper; he also delivered lectures at the British Association for the Advancement of Science annual meeting in Belfast, a meeting of the British Working Group on Integrated Control in Fruit Pests, and a Royal Society Discussion Meeting entitled 'Biological Control of Pests, Pathogens and Weeds: Developments and Prospects', after which he attended a meeting organized jointly by the Royal Society and the Ciba Foundation entitled 'Challenges in Biological Control'. In addition, he gave a number of talks to universities, colleges, government-sponsored and industrial research institutes and private societies. Papers were also presented by P.H. Nicholls at the British Crop Protection Conference in Brighton and K. Chamberlain at the meeting 'Studies in Pesticide Transfer and Performance' organized by the Association of Applied Biologists. B.J. Pye attended a meeting organized by Ciba-Geigy on 'Science, Sprays and Sprayers' and I. Denholm and M.W. Rowland attended the winter meeting of the British Ecological Society, 'Insect Population Ecology'. Several staff attended a meeting on 'Neuropeptides' at the Society of Chemical Industry, organized by J.A. Pickett who continues as chairman of the Pesticides Group of the Society of Chemical Industry. He also chairs the Mid-Anglia Section committee of the Royal Society of Chemistry. J.A. Pickett and P.H. Nicholls acted as chairman and technical secretary respectively on two of the sub-groups of the Crop Protection Research Consultative Committee, set up by the AFRC to report to the Priorities Board for Research and Development in Agriculture and Food.

PLANT PATHOLOGY DEPARTMENT

The Department continues to attract funding from external sources as diverse as the Agricultural Genetics Company and The Commonwealth Universities. There are now 10 Ph.D. students in or associated with the Department and all programmes have additional support. This year also saw the establishment of the first Linked research group, with Cambridge University on the take-all disease of cereals. Regrettably core funding continues to decline and it is likely that two more posts will be lost in the next financial year.

Evidence of our work on diagnostic methods is given in the individual reports below. This now covers viruses, fungi and bacteria and will be strengthened by the setting up of a monoclonal antibody unit within Plant Pathology which should be functioning early in 1988. Although Plant Pathology will be a major user it will be available as a resource to the Institute as a whole.

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Diseases of break crops

Oilseed rape

Cultivars, spray timing and disease. Fungicides are commonly applied twice to winter oilseed rape crops, usually in autumn or spring to control light leaf spot (*Pyrenopeziza brassicae*) and in summer against dark pod spot (*Alternaria* spp.). Experiments in 1985-7 tested the effectiveness of a single spray of prochloraz in November or April or iprodione in June and all combinations of these treatments on cultivars Jet Neuf, Bienvenu, Darmor, Liradonna, Mikado, Rafal and Ariana, although not all cultivars were used in each year. Diseases were common but not severe in 1985 and 1986 but in 1987 infection by *P. brassicae* was severe and *Alternaria brassicae* was common.

The autumn application of fungicide contributed most to control of *P. brassicae*, although a further small, but usually insignificant, decrease of infection was given by the spring treatment. The effect of the autumn application was still evident eight months later even though there was no visible infection at the time of treatment. Cultivars responded differently but in 1985 and 1986, when *P. brassicae* was the principal pathogen, the best yields were from plots treated in autumn and spring. In 1987, when *A. brassicae* was prevalent and severe on earlier maturing cultivars the best yields resulted from autumn and summer treatments.

Substantial yield increases resulting from fungicide treatment were not always clearly associated with disease incidence. For example, an increase of 0.9 t ha^{-1} (3.8 to 4.7 t ha^{-1}) in cv. Bienvenu from autumn + spring + summer applications in 1985 was associated with decreased shedding of seed in sprayed plots. This was especially important because prolonged wet weather delayed harvest. In 1987, when *P. brassicae* was severe from February onwards, autumn and spring applications were associated with an increase in the number of fully expanded leaves per plant in late April. Effects on dry matter and leaf area are noted elsewhere (see p. 120 Crop Production Department). There was more *P. brassicae* on leaves free of *Peronospora parasitica,* suggesting an interaction between these pathogens.

The importance of correctly timed applications of fungicide to control *P. brassicae* is increased now that the sexual stage has been found (*Transactions of the British Mycological Society* (1987) **89**, 135-140) on cultivars other than Jet Neuf (see below). The occurrence of apothecia on the newly recommended low glucosinolate cultivar Ariana, not grown here previously, indicates that long adaptation of the fungus to one susceptible cultivar (e.g. Jet Neuf) is not a prerequisite for the formation of the sexual stage. (Rawlinson; Church, Inman and C. Wilson)

Release and dispersal of ascospores of *Pyrenopeziza brassicae*. Investigations of the physics of spore dispersal led to the discovery of the sexual stage of *P. brassicae*, the cause of light leaf spot on oilseed rape, in cv. Jet Neuf in 1986 (*Rothamsted Report for 1986*, 51). Jet Neuf is no longer recommended, but, in May and June 1987, apothecia were identified on plant debris from unsprayed plots of the currently-listed cultivars Ariana, Bienvenu, Primor and Liradonna. Ascospores were also collected from debris of two other recommended cultivars, Rafal and Mikado. The presence of the sexual stage of *P. brassicae* on a wide range of cultivars has an important influence on the epidemiology of light leaf spot in the UK oilseed rape crop.

Volunteer seedlings growing in unploughed rape stubble after harvest in 1986 developed light leaf spot symptoms by September and had small and large apothecia on dead leaves by October. A plot inoculated with infected straw in late October showed symptoms by the end of November; apothecia were first noted in January and by May were present on debris from nearly all plants. Field observations show that it takes between two and three weeks for apothecia to develop on infected dead leaves. In the laboratory experiments showed that

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dead plant tissue containing apothecia must be wet before ascospores are released and that these spores will continue to be released for up to a week if the material remains wet. Thus, in the field, ascospore release should continue for two or three days following rain if plant debris does not dry out. Ascospores have been observed in the field during much of each season and in large numbers periodically from March until July in both 1986 and 1987.

These observations suggest that the sexual stage of *P. brassicae* can become common in susceptible oilseed rape crops and that it may be important in disease spread within the crop, especially after flowering when large numbers of ascospores can be released. Further, as the disease can develop quickly on volunteer seedlings, unploughed stubble or headlands could act as sources of inoculum for succeeding crops. Indeed, in 1987 ascospores were trapped over an uninoculated plot of oilseed rape as early as October. (McCartney; M.E. Lacey, Crop Production)

Sunflower

Diseases, maturity and yield. Despite the unfavourable, cold, wet weather in 1987 four out of six cultivars sown on 17 April reached maturity and were harvested on 25 September; the best yield was 2.55 t ha^{-1} at 90% DM. The wettest October on record and severe winds ensured that later harvested cultivars yielded well below their full potential.

Head infection by *Botrytis cinerea* became severe near maturity in most varieties. Carbendazim (as 'Bavistin FL' at 0.5l ha^{-1}) and vinclozolin (as 'Ronilan' at 1l ha^{-1}) controlled stem infection but five sequential sprays, beginning at mid-flowering and repeated every two weeks, only gave temporary control of incidence and severity on heads, and electrostatic spraying was no more effective than hydraulic. Removal of disk florets after anthesis slightly lessened subsequent infection on the head which suggests that senescing florets may be an important infection site.

Alternaria alternata was isolated from leaf lesions on a few plants with symptoms identical to those caused by *A. helianthi*. *Sclerotinia sclerotiorum* destroyed the heads of a few plants, too few to test control measures. A deformity of heads, in which secondary ray florets formed at the centre of flowers, was widespread, up to 31% in one cultivar, but was not associated with a virus or mycoplasma. The cause may have been an interaction between genotype and low temperatures during floral initiation. (Rawlinson; Church, Inman and C. Wilson, with Jones, Turnell and Gordon, Field Experiments)

Grain legumes

Pea seed-borne mosaic virus (PSbMV). The detection of PSbMV in pea seed by indirect enzyme-linked immunosorbent assay (ELISA) was improved by the addition of cellulase (0.1%) or 'Triton X-100' (0.1%) to the extraction fluid. The additives, separately and together, increased the A_{405} values of infected seed extracts, presumably because they aided the release of virus particles from host materials.

In standardized tests, with 'Triton X-100' in the extraction fluid, PSbMV was detected in 23 of 26 seed lots of cv. Waverex, with 70–95% of seeds infected in some samples. In subsequent growing-on tests, 0–8 and 5–24% infection was detected in seedlings grown from seed lots with, respectively, 1–50 and 50–95% infection.

In glasshouse studies, in which seeds from plants that had been inoculated at the seedling stage were grown on, there was much transmission of the virus through seeds of cvs Waverex (19%), Birte (48%) and Vedette (87%), but none through seeds of cvs Maro and Progreta. There was also no transmission through seeds from Progreta plants that were naturally infected with PSbMV, presumably through transmission by aphids, in the field.

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In field studies, foliar sprays of pirimicarb and the pyrethroid PP321 applied before, during and after flowering to peas sown at the end of April decreased aphid numbers but seemed not to affect the spread of PSbMV. At the end of flowering the mean incidence of PSbMV in unsprayed and sprayed plots of cv. Waverex, with 8% seed-borne infection, was 89 and 81% respectively; corresponding results for adjacent plots of Waverex grown from uninfected seed was 19 and 19%, and for adjacent plots of cv. Progreta, also grown from uninfected seed, 29 and 20%. (Cockbain; Ding and Wang, with Mr. A.J. Biddle, Processors and Growers Research Organisation)

Vicia cryptic virus (VCV). Indirect ELISA proved to be as reliable as immunospecific electron microscopy (ISEM) for detecting VCV in *Vicia faba* and was used to screen seeds for infection. Infected leaf extracts from small- and large-seeded cultivars of British or Chinese origin gave similar A_{405} values, suggesting that the viruses in the different cultivars were antigenically similar or identical. VCV was not detected in six accessions of *V. faba paucijuga*, nor in the distantly related *V. bithynica, V. galilaea, V. johannis, V. narbonensis* and *V. serratifolia*. (Cockbain; Smitamana and Woods)

Fungus diseases of lupins. In 1986 and 1987 a large proportion of the plants of *Lupinus albus* grown at Woburn and sown early (17 March 1986, 2 or 23 February 1987) died prematurely (*c.*20, 80 and 50% respectively). Plots at Rothamsted suffered less than those at Woburn, where later sowings survived better than those sown early. There were occasional well-grown but wilted plants at both sites. *Fusarium* spp. were frequently isolated from the roots and stems of dead or wilted plants but were also isolated from some apparently healthy plants. A *Fusarium* sp. was isolated in August 1987 from the withered tips of shoots on some plants at Woburn.

A *Botrytis* sp. was consistently isolated from lesions on pods at Woburn in 1986 but generally leaf pathogens were few. In 1987, rust (*Uromyces* sp.) was common at Rothamsted in September. (Jenkyn; Creighton with Yeoman, Field Experiments)

Fungal invasion of lupins following desiccation. In 1986, because of wet weather, harvest of lupins cv. Vladimir was delayed until 9 December, eight to 11 weeks after desiccant was applied. Consequently, the seeds were of poor quality and many were discoloured or carried visible fungal mycelium. Less than 15% of the harvested seed germinated although 30% appeared free of blemishes; more blemishes occurred in seeds treated with the higher rate of diquat (3 l 'Reglone' ha^{-1}) than those receiving other treatments. Blemishes were fewer when desiccant was applied after all leaves had fallen (15 October), rather than earlier, especially when propiconazole (0.5 l 'Tilt' ha^{-1}) had been applied about two weeks previously; fewest blemishes were on seeds not treated with desiccant, whether or not they received fungicide. The most frequently isolated fungi were *Stemphylium* spp., *Alternaria* (including *A. alternata, A. tenuissima* and the *Alternaria anamorph* of *Pleospora infectoria*), *Botrytis cinerea, Phomopsis* sp. and *Fusarium* spp. (including *F. avenaceum* and occasional *F. culmorum*) but neither fungicide nor desiccant treatment consistently affected their incidence or relative proportions. (Lacey; Nabb, with. H. Jones, Field Experiments)

Cereal diseases

Eyespot

Characterization of pathotypes. The identification of pathotypes of the eyespot fungus (*Pseudocercosporella herpotrichoides*) by colony morphology on agar plates is difficult and

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inaccurate; cultures isolated from lesions may be mixtures or apparent intermediates between wheat (W) and rye (R)-types. However, when isolations are made on an agar medium based on maize meal and exposed to near ultraviolet light, marked differences in pigmentation occur. R-type isolates produce pink/red/brown pigments, whereas W-type isolates produce black pigments. In collaborative studies the potential of monoclonal antibodies and isoelectric focusing to help diagnosis of eyespot and separation of R- and W-pathotypes are also being investigated.

Pathogenicity of pathotypes. Sixteen English and 16 French isolates of *P. herpotrichoides* were compared in controlled environments at Rothamsted and Rennes. Few differences in pathogenicity between isolates from the different countries were detected. Both W- and R-type isolates were more pathogenic to wheat than rye, W-type isolates were slightly more pathogenic to wheat than R-type isolates, but R-type isolates were slightly more pathogenic to rye.

Population studies. Some plots of an experiment started in 1984 (*Rothamsted Report for 1985,* 124) were treated with fungicides in November 1985, April and November 1986, and April 1987. Samples taken in April and July 1986 and 1987 showed that carbendazim did not decrease the incidence of eyespot. Prochloraz was effective but prochloraz plus carbendazim decreased incidence most. The incidence of eyespot was greater in July than April in both years. In 1985, treatment with carbendazim plus prochloraz increased yield from 6.5 t ha^{-1} to 7.4 t ha^{-1} but no treatment increased yield in 1986; no yields were taken in 1987.

The proportions of R-type and MBC-resistant isolates in untreated, uninoculated plots continued to increase in 1986. In plots treated with carbendazim more than 95% of isolates were MBC-resistant. However, in untreated plots originally inoculated with MBC-resistant populations the proportion of MBC-resistant isolates declined, the decrease apparently occurring between seasons. Although few isolates were recovered from plots inoculated with W-type isolates and treated with prochloraz the proportion of R-types isolated increased.

Pseudocercosporella anguioides. *P. anguioides* was isolated from four out of over 2500 suspected eyespot lesions on wheat collected from a single field in April 1986, and from eight out of over 1500 such lesions taken in April 1987. The fungus was not isolated from stem lesions sampled in July. The method of isolation, involving washing conidia from lesions incubated under near-ultraviolet light, is likely to underestimate the incidence of this fungus. The isolates taken in 1986 were not pathogenic to wheat seedlings in pots, but were re-isolated from some symptomless leaf sheaths taken from the inoculated plants.

Disease development. The development of eyespot was followed in plots of winter wheat, cv. Avalon, and winter barley, cv. Opera, sown at 200 and 400 seeds m^{-2} (wheat) or 150 and 300 seeds m^{-2} (barley) inoculated or not with W-type or R-type isolates of *P. herpotrichoides*. In April, the incidence of lesions on leaf sheaths was greater in inoculated than uninoculated plots but was unaffected by pathotype. Fewer shoots were infected at the lower (barley 75%, wheat 66%) than at the higher seed rate (barley 86%, wheat 91%). By mid-June there were fewer shoots of wheat (30%) with moderate or severe lesions than of barley (50%). In barley, 78% of shoots in plots inoculated with W-type isolates and 33% of shoots in plots inoculated with R-type isolates, had moderate or severe lesions; seed rate had no effect. In wheat, seed rate had little effect on the incidence of severe or moderate lesions in plots inoculated with W-type isolates. However, in plots inoculated with R-type isolates eyespot was more severe at the high seed rate (31% severe or moderate lesions) than

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at the low seed rate (14%). Delaying the application of prochloraz plus carbendazim from Zadoks growth stage (GS) 30–31 the start of stem extension, to GS 37–39, flag leaf emergence, increased yield on plots inoculated with R-type isolates. (Fitt; Bateman, Goulds, Creighton with Robinson, Entomology and Nematology, Dr. M. Dewey, University of Oxford, and Mme N. Cavelier, Rennes)

Leaf blotch

Epidemiology. The latent period of leaf blotch (*Rhynchosporium secalis*) on winter barley (cv. Maris Otter) in controlled environment experiments with relative humidity close to 100% and a range of temperatures was 24–37 days at 5°C, 19–25 days at 10°C, 15–20 days at 15°C and 13–16 days at 20°C. In all experiments some inoculated leaves did not develop lesions before they senesced. Most of these leaves were found to have unapparent infections which were often sporulating. (Davis; Fitt)

Take-all

ELISA and the take-all fungus. ELISA tests using an antiserum already produced against fungi believed to be *Gaeumannomyces graminis* var. *tritici (Ggt)* discriminated amongst fungi closely related to *Ggt*, fungi distantly related to *Ggt* and unrelated fungi (*Rothamsted Report for 1986*, 119). Fresh antisera are now being prepared against known *Ggt*. As well as antigenic preparations of whole fungus, cell wall preparations are also being used to try to produce higher titres and greater specificity. Antiserum prepared against the whole fungus antigen has once again given encouraging results and it has distinguished between *Ggt* (strong reaction), *G. graminis* var. *graminis* and *Phialophora* sp. lobed hyphopodia (intermediate reaction) and *Colletotrichum coccodes* (weak reaction). Results with cell wall antigen are so far disappointing. It is hoped to increase antiserum specificity further by cross-absorption techniques and to use this antiserum for the purification of *Ggt*-specific, enriched antigen suitable for the production of monoclonal antibodies. Such materials would further taxonomic and ecological studies of the pathogen and diagnosis and quantification of the disease. (Hornby; R.F. White, J.A. White, MacKenzie)

Barley yellow mosaic virus (BaYMV)

Strains. The second strain of BaYMV, identified from Wiltshire in 1986, (*Rothamsted Report for 1986,* 119) was purified and an antiserum prepared. Immunospecific electron microscopy (ISEM) tests on field samples sent by ADAS plant pathologists showed that both strains were widespread either singly or as mixed infections. The Wiltshire strain could be transmitted mechanically but not very efficiently and only if inoculated plants were kept below 15°C, whereas the first (Streatley) strain has an optimum temperature of 20–23°C. Immune barley cultivars seem not to be infected by either virus strain but amongst other cultivars there may be some differences in relative susceptibility to the two strains.

In experiments preliminary to the production of cDNA clones of BaYMV, one isolate of the virus has been purified and the RNA isolated. Two lengths of RNA are regularly obtained and *in vitro* translation studies have been started. (Adams; Antoniw, Jones, Swaby, Batista with Dr. K.R. Wood, Birmingham University)

Oat golden stripe virus (OGSV). This soil-borne virus occurs occasionally in the UK, often in association with oat mosaic virus, and is presumed to be transmitted by the same fungal vector *Polymyxa graminis*. The virus was maintained in oats by mechanical inoculation

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and purified by extraction in 0.5M borate buffer, two cycles of centrifugation through sucrose cushions and isopycnic centrifugation in CsCl. The particles had median lengths of 150 and 300 nm, sedimented as two components of 168 and 218 *S* and had a buoyant density of 1.321 g cm^{-3}. An antiserum was prepared and used in ISEM and ELISA tests in comparison with antisera to other members of the furovirus group. OGSV had some affinities with soil-borne wheat mosaic virus but not with beet necrotic yellow vein, hypochoeris mosaic or potato mop-top viruses. (Adams; Swaby, Jones)

Barley yellow dwarf virus (BYDV)

Strain identification. During 1986 and 1987, 782 dried cereal leaf samples (wheat, barley, oats and maize) from 16 countries were tested by ELISA for BYDV. PAV, RPV and MAV-like serotypes were identified with three strain specific monoclonal rat antibodies (produced by Dr. L. Torrance, MAFF Harpenden Laboratory) in an indirect ELISA using a mixture of broadly specific polyclonal anti-BYDV rabbit antisera to trap the viruses.

Of all samples received 68% were positive in the ELISA but 9.5% could not be assigned with confidence to any of the three serotypes. Samples from North Africa, Middle East and Pakistan had most (22%) unassigned positives. There were also regional differences in the proportion of samples received that were positive. However, it is uncertain whether this reflects differences in serotype or inexperience of the collector in assessing symptoms. It is also possible that isolates, such as RPV, that cause relatively slight symptoms might be under represented in samples based on symptoms. Two-thirds or more of the samples from Central and South America, North Africa, Middle East and Pakistan, half of those from East Africa and New Zealand, and a quarter of those from China and Nepal, were positive. Most of the isolates (50%) were MAV-like, 30% were PAV-like and 6% RPV-like but there were large regional differences. The predominant isolates in Central and South America and East Africa were MAV-like (57−67%), 21−27% were PAV-like and 0−7% RPV-like. From North Africa, Middle East and Pakistan 57% were PAV-like and only 4% MAV- and RPV-like. No MAV-like samples were detected in New Zealand samples of which 78% were PAV-like.

Immunospecific electronmicroscopy. The three strain specific monoclonal antibodies were compared with two polyclonal antisera in ISEM tests. When used at 1 μg ml^{-1} the monoclonal antibodies specifically trapped the MAV-, RPV- and PAV-like isolates, whereas the polyclonals showed considerable non-specific reaction. This is the first report of the successful use of monoclonal antibodies for diagnosing BYDV strains in plant sap by ISEM.

Resistance to BYDV in barley. Although resistance to BYDV conferred by the Yd_2 gene has been known and used for some years in spring barley cultivars elsewhere in the world, cultivars with this resistance have not been recommended for use in Britain. However, in 1987, the winter barley cultivar Vixen, into which the Yd_2 gene has been transferred, was added to the recommended list especially for use where BYDV is a frequent problem.

Vixen is closely related to the widely grown winter barley cv. Igri and the BYDV isolate B, a stable mixture of BYDV isolates PAV and RPV, was inoculated to both cultivars on four occasions (September, October, April and May). The September inoculation killed Igri and decreased the yield of Vixen by more than 90%, inoculation in October decreased the yield of Igri by 95% and Vixen by 80%. Inoculation in the spring was less damaging, the yield of Igri was decreased by 20% and 5% with April and May inoculations respectively and the yield of Vixen by 12% and 6%. On this evidence the resistance shown by Vixen is insufficient to make the use of insecticidal control unnecessary.

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However, when a further experiment was done using single strains of BYDV isolated at Rothamsted to inoculate Igri and Vixen in autumn or spring, different results were obtained. Autumn inoculation of Vixen by MAV or PAV did not decrease yield, but 1000 grain weight and height were decreased and shoot number increased. Inoculation by strain RPV decreased yield by 90%, halved plant height and decreased 1000 grain weight, grains/ear and shoot number substantially. All strains decreased yield of Igri, strains RPV and MAV decreased yield by 90% and 60% respectively, strain PAV killed all plants.

In spring, inoculation of Vixen by strain MAV had no effect on yield but, surprisingly, PAV decreased yield by 20% largely by decreasing 1000 grain weight, an effect which was not compensated for by an increase in shoot number. Strain RPV decreased yield by 35%. On cv. Igri PAV was again the most damaging strain decreasing yield by almost 50%. Strain MAV was almost as damaging (−42%) and RPV least damaging (−25%).

These results suggested that while the Yd_2 gene was effective against PAV- and MAV-like isolates it had little or no effect against RPV-like isolates. This was supported by results from tests on the near-isogenic spring barley cvs Atlas 57 ($-Yd_2$) and Atlas 68 ($+Yd_2$) inoculated separately with the three isolates. The presence of the Yd_2 gene prevented much of the damage caused by PAV- and MAV-like isolates but conferred little or no benefit when the RPV-like isolate was used.

Infectivity testing and forecasting. The risk of BYDV infection, as measured by the Infectivity Index, was much lower in 1986 (12) compared with 1985 (95). Aphid numbers on barley plots sown on 12 and 22 September and 1, 10 and 24 October 1986 remained low, 0.33, 0.17, 0.18, 0.01 and 0.01 aphids per plant respectively, throughout the autumn and winter and there was negligible virus infection. There was no effect of sowing date or cypermethrin on yield, thus confirming the prediction given by the Infectivity Index. (Plumb; Barker, Forde, Herrera, and Lennon, with Carter and Morgan, Entomology and Nematology)

Potato diseases

Susceptibility of cultivars to black dot *(Colletotrichum coccodes).* In 1985 and 1986, seed tubers of five and six cultivars respectively were planted in experiments at Rothamsted and Woburn. From both farms in both years tubers of cv. Désirée had the most disease. Although it is known that soil inoculum is important in the epidemiology of this disease, differences in amounts of disease between cultivars may have been influenced by differing amounts of seed-borne inoculum. In 1987, seed tubers of 12 cultivars were planted in an experiment at Rothamsted. Tubers without black dot were selected and treated with prochloraz before sprouting but half the plots were inoculated with cultures of *C. coccodes* at planting. After harvest black dot was prevalent in non-inoculated plots (mean disease rating 29) but had been increased by inoculation (44). With most cultivars inoculation increased the disease rating by *c.* 20 but the increase was much less in King Edward (3), Record (5) and Cara (8). Désirée (59), Maris Piper (53) and Record (47) had most disease and tubers of Cara (13), Pentland Crown (22) and Romano (25) least. These results suggest that the risk of losses in crop quality caused by black dot differs with cultivar. (Read; Hide)

Potato virus diseases at Rothamsted. When counts were made in late June, plots planted with Pentland Crown seed grown at Rothamsted in 1986 were free from infection with the 'severe' viruses; Désirée had 0.5% leaf roll and Maris Piper 0.02%; King Edward had 0.2% potato virus Y. Conditions were ideal for expression of symptoms of potato mop-top virus.

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Aphids *(Myzus persicae)* were scarce in 1987 and no virus spread was detected to seed crops. (Govier)

A soluble dye, blot immunobinding assay for virus detection in potatoes. A technique has been developed in which potato sap is sampled by squashing squares (*c.* 1 cm^2) of a protein-absorbent nylon membrane between leaves or in a slit cut in a tuber. The nylon squares are dried, thus binding any virus particles firmly to the membrane. Remaining protein-absorbent sites are blocked by incubating the squares in a casein solution. The membrane is then probed with a virus- specific antibody to the test virus linked directly or via a second antibody to the enzyme alkaline phosphatase. The presence of virus is revealed by incubating the squares in the substrate nitrophenyl phosphate, which turns yellow in the presence of the enzyme. Results can be assessed visually or by measuring the A_{405}.

The technique clearly differentiated between uninfected potato plants and plants infected with either potato leafroll virus (PLRV) or potato virus Y (PVY). In a replicated experiment, the A_{405} given by PLRV-infected leaf and tuber samples were 1.49 and 0.90 respectively, whereas for corresponding uninfected samples they were 0.29 and 0.07 (SE = 0.094). Similarly, A_{405} for PVY infected leaf and tuber samples were 2.20 and 2.00 whereas those of uninfected samples were <0.03.

This technique is simple, sample preparation is easy, and might be done by farmers. Processing involves fewer steps than ELISA and assay can be by eye. (Gibson)

Viruses

Cryptic viruses. Clones to dsRNA of beet cryptic virus (BCV) were produced, so that cDNA clones to all four dsRNA components of the two cryptic viruses in beet are now available. Hybridization studies showed that there is no significant homology between these four dsRNAs or with the dsRNA of ryegrass cryptic virus (RCV) or fescue cryptic virus (kindly supplied by Dr. G.P. Accotto, Turin). Clones of RCV were isolated from a cDNA library and are being characterized. (Antoniw with Dr. E.P. Rybicki, University of Cape Town)

Viruses of tropical crops. Further work on the yellow mosaic disorder of sweetmelon and watermelon crops in the People's Democratic Republic of Yemen showed that the principal cause appears to be a graft-transmissible geminivirus, not melon rugose mosaic virus as was previously thought (*Rothamsted Report for 1985,* 129). Studies of putative vectors of the geminivirus showed a positive correlation between populations of the whitefly *Bemisia tabaci* and disease incidence. The geminivirus is serologically related to squash leaf curl virus (Dr. B.D. Harrison, personal communication) but other characters differ. (Jones, with M.H. Sattar and N. Al-kaff, PDR Yemen)

Fastidious prokaryotes

Sumatra disease of cloves. Repeated transmission tests confirmed that tube-building cercopoids, *Hindola fulva* (in Sumatra) and *H. striata* (in Java) are vectors of Sumatra disease. *H. striata* acquired the Sumatra disease bacterium (SDB) during 4 hour but not 30 minute acquisition-access periods on diseased plants, and transmitted bacteria within 49 hours of acquisition. The efficiency of acquisition increased with increasing acquisition-access periods but differed greatly when plants showing similar symptoms were used as sources.

Most of the main clove growing regions of western and North Sumatra are now affected by the disease; *H. fulva* is distributed throughout these regions. Outbreaks of the disease

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Have been confirmed in Central Java, at least 400 km from the nearest known source of infection. Representative isolates of bacteria from surveys provided a nucleus for studies on the characterization of the SDB.

In pathogenicity tests, representative isolates of SDB were compared with a collection of cultures of *Pseudomonas solanacearum* that had occasionally been isolated from clove plants. Isolates of SDB invaded the vascular tissue of clove seedlings but did not cause symptoms in solanaceous hosts, whereas fluid colonies of all isolates of *Ps. solanacearum* gave typical symptoms in solanaceous hosts but showed only limited pathogenecity to clove seedlings. These differences support the view that the two organisms are taxonomically distinct. (Eden-Green, with staff from the Research Institute for Spices and Medicinal Crops, Bogor, Indonesia)

Many isolates of the SDB and *Ps. solanacearum* and a range of other plant pathogenic bacteria were examined using standard bacteriological tests. The resulting data were used to construct a similarity matrix and used in hierarchical cluster and principal coordinate analyses. Although SDB was clearly separated from *Ps. solanacearum* and other plant pathogenic bacteria it was only on the basis of negative test results, which may be of use for presumptive identification but are of little value in confirmatory diagnosis.

The specificity of rabbit polyclonal antisera to SDB and *Ps. solanacearum* was improved by affinity chromatography, using an SDS-bacterial cell extract immobilized on Sepharose 4B, and cross absorption using whole bacteria in suspension. The improved antisera, especially that to SDB, are of adequately high titre and specificity to permit identification and discrimination between SDB and *Ps. solanacearum* using ELISA. Their use has suggested that serotypes of SDB may exist. (Ambler and S.J. Roberts)

Biodeterioration

Detection of moulding in cereal grains. Fungi colonizing cereal grains present hazards to the health of workers handling them and to humans and animals consuming them as well as decreasing quality through the action of hydrolytic enzymes. Enzyme production by common storage *Aspergillus* and *Penicillium* species was studied using chromogenic 4-nitrophenyl substrates. A method was developed for the spectrophotometric assay of enzyme production utilizing a microtiter plate reader, enabling the rapid assay of many samples using small amounts of enzyme extracts. Of the enzymes assayed, including esterases, glycosidases, lipases, phosphatases and sulphatases, β-D-glucosidase, α-D-galactosidase, N-acetyl-β-D-glucosaminidase, acid phosphatase and aryl sulphatase occurred in larger amounts in mouldy than in clean grain. In particular, N-acetyl-β-D-glucosaminidase was indicative of *Aspergillus amstelodami* colonization. (Lacey and Jain).

Aerobiology

Spore dispersal in splash droplets. A model to describe the incorporation of spores of *Rhynchosporium secalis* (barley leaf blotch) into splash droplets has been developed based upon data from experiments in which simulated rain fell on to either infected leaves or straw. The model indicates that a) spores are removed from host surfaces by the large radial shear forces produced during the impact of a raindrop; b) spores mix with water from the raindrops and from the host surface in such a way that the spore concentration changes with time during a splash, the total time of which is <1ms for a thin film <1mm deep and c) small fast-moving droplets are produced near the beginning of the splash and large slow-moving droplets towards the end. Experimental data show this is equivalent to representing the number of spores per droplet (n) plotted against droplet diameter (d) by a family of parallel lines of constant

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concentration, i.e. n proportional to d^3. Further work is reported by the Crop Production Department). (Fitt with Walklate and McCartney, Crop Production)

Airborne microorganisms associated with domestic waste disposal. To supplement a recently-completed study of airborne microorganisms in 15 domestic waste handling facilities, personnel handling waste at each site completed a questionnaire to determine whether concentrations of airborne fungi and bacteria were associated with any health problems. Of 150 exposed personnel, 30% reported symptoms of fever, influenza-like chills and diarrhoea, perhaps related to Gram-negative bacterial endotoxin exposure, but none of these symptoms were reported by an unexposed group. About 26% of both exposed and control groups reported respiratory symptoms, but these may have been smoking-related, since smokers comprised 82% of this group, compared with 46% among symptomless workers. It was concluded that incidence of allergic lung disorders from exposure to airborne fungi may not be great in waste handling facilities but symptoms may arise from exposure to airborne Gram-negative bacteria which were abundant in the waste sites studied. Workers should therefore wear respiratory protection in these areas.

Assessment of filter samples from working environments. Scanning electron microscopy, light microscopy, epifluorescence microscopy and culture were compared as methods for assessing the catch on membrane filters exposed in polypropylene holders in different working environments heavily contaminated with airborne microorganisms but differing in the relative abundance of bacteria, actinomycetes and fungal spores. Except in pig houses, estimates by scanning electron microscopy and light microscopy were similar and those by fluorescence microscopy were smaller. However, fluorescent staining enabled bacteria on air-borne pieces of skin in pig house samples to be counted. Estimates by culturing sometimes exceeded those obtained by fluorescence microscopy but were always smaller than those obtained by other methods. From 0.1 to 68% of bacteria + actinomycetes and 3−98% of fungal spores seen by light microscopy were accounted for by colonies grown in culture.

Deposition on the filter surface was not uniform, necessitating a systematic distribution of counting fields when filters were examined directly by light or scanning electron microscopy. Deposition was more uniform when electrically conducting graphite-filled-polypropylene filter holders were used open-faced. Losses within filter holders and during transportation from sampling site to laboratory were small and counting precision averaged 26% for total spores although it was less for single spores and for aggregates. To achieve a relative precision >10%, at least 200−300 fungal spores and 500 bacteria + actinomycetes (150−2000 spore containing particles) should be counted.

Detection of airborne allergens. Air samples were taken at the start and finish of an experiment in composting degradable fractions of domestic waste out of doors to assess the concentrations of airborne microorganisms that may be inhaled by workers handling the waste. The composting process led to a thousand-fold increase in numbers of thermophilic bacteria, actinomycetes and fungi released into the air when the waste was disturbed. Workers were exposed to mean concentrations 10^6 colony-forming units (CFU) of *Aspergillus fumigatus* spores m^{-3} and $>10^7$ CFU thermophilic actinomycete spores m^{-3}, despite rapid dispersal of the spores by wind.

In pig confinement units where typical concentrations of airborne particles ranged from 6×10^6 to 4×10^7 m^{-3} depending on design, ventilation and activities in each unit. Most particles were small enough to allow potential penetration to alveolar regions of the lung. Airborne fungi and thermophilic actinomycetes were relatively few but bacteria, mostly Gram-positive cocci associated with desquamated skin cells, were abundant.

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Work-related respiratory symptoms have been reported among workers using pressurized water jets to shell prawns in seafood processing factories. Preliminary laboratory studies using aerosols of prawn protein solution showed that recovery of material deposited directly into liquid, a novel technique for airborne antigen collection, gave better recovery than collection by filtration onto PTFE or glass fibre filters. (Lacey; Crook, Williamson, J. Wilson, Higgins with Dr. W. Eduard, (National Institute of Occupational Health, Oslo, Norway), Dr. K. Karlsson, (National Board of Occupational Safety and Health, Solna, Sweden), Dr. U. Palmgren, (Swedish University of Agricultural Sciences, Uppsala, Sweden), Dr. G. Ström, (National Board of Occupational Safety and Health, Umea, Sweden), Dr. M.D. Topping, Dr. P. Griffin, Miss S. Travers (Health and Safety Executive), Dr. P. Bardos, (Warren Spring Laboratory), Dr. J. Robertson, (Scottish Farm Building Investigation Unit, Aberdeen).

Staff and visitors

Dennis Govier retired after 20 years in the Department and Sally Higgins, Steven Roberts, Tracy Feekins and Helen Stuart resigned. John Antoniw joined the Department in January following transfer from the Biochemistry Department and Douglas Henry, Catriona MacKenzie, Vicky Martin, Andrew Hackett and Kevin Doughty joined. Toni Fleming, Fatima Batista, Nannapaneni Ramakrishna, Dao-Wen Wang and Martin Holtum began Ph.D. work in, or associated with, the Department.

Members of the Department made many visits overseas to further collaborative projects or to attend and contribute to international conferences. At the request of the Overseas Development Administration, R.D. Woods and P. Jones organized a workshop in Nairobi, Kenya on virological methods, especially electron microscopy. P. Jones also visited the ODA funded project in Zanzibar on the 'sudden death' disease of cloves and the People's Democratic Republic of Yemen to advise on the possible aetiology and control of a yellowing condition of melons. B.D.L. Fitt visited and lectured at Research Institutes and Universities in the United States and Canada, J.F. Antoniw contributed to the 7th International Congress of Virology in Edmonton, Alberta. J. Lacey visited Sweden and Norway, funded by the Nordic Ministerial Council, to discuss collaborative work on air sampling methods, he also visited The Pasteur Institute and attended a meeting in Paris on the hazards of moulds in the agriculture and food industries. There were several visits by French workers to Rothamsted and G.L. Bateman, B.D.L. Fitt, A. Goulds, R.J. Gutteridge and N.F. Creighton visited France to further co-operative programmes on eyespot and take-all, supported by the French Government through their embassy in London. R.W. Gibson attended a workshop in Wageningen which discussed diagnostic methods for plant pathogens, R.T. Plumb, I. Barker, S.M.D. Forde and G.H.M. Herrera attended, and contributed several papers and posters to a meeting on barley yellow dwarf virus, sponsored by the International Maize and Wheat Breeding Centre, Mexico, which also provided funds. C.J. Rawlinson contributed to a meeting in Warsaw on oilseed rape.

Visitors who spent different periods working in the Department came from Kenya, Cameroons, USA, France, India, Thailand, PDR Yemen, United Arab Emirates, People's Republic of China, Republic of South Africa, Brazil and Chile.

J. Lacey and D. Hornby continued as Chairmen of Committees of the International Society for Plant Pathology and have been very involved with preparations for the International Congress in Kyoto in 1988. Several other members of the Department play an active role in scientific societies as convenors, members of Council and editors. We are grateful to the Overseas Development Administration, MAFF, Potato Marketing Board, Health and Safety Executive, Bayer, BASF, Schering, The Agricultural Genetics Company (De Danske Sukkerfabrikker) and The Hills Bequest for financial support.

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