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Iacr Report for 1998

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Agricultural Systems

Rothamsted Research

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Agricultural Systems

R.T.PLUMB

Understanding the variability of pests, pathogens and their hosts, and their occurrence and distribution, is central to the implementation of effective methods of managing them in agricultural systems. New developments in analysis, crop protection products, information technology, crops and the occurrence of 'new' constraints to yield, constantly need integration into existing farming systems. This year's report considers some of these developments, including sporadic pests of sugar beet, recent developments in understanding the take-all disease of cereals and in forecasting light leaf spot of oilseed rape, the use of willows for short-rotation coppice and their pests and diseases, and novel methods of measuring non-randomness.

SPORADIC PESTS IN SUGAR BEET

Forecasting is an integral part of many pest management systems. However, some pests are so uncommon that they are impossible to predict, largely because they have not occurred often enough to collect meaningful data on the factors affecting their abundance. There have been several pest outbreaks in sugar beet which typify this situation, for example thrips in 1996 and 1997, caterpillars of the silver Y moth in 1996, and two-spotted spider mites in 1995 and 1996. To learn more about these pests, some opportunistic research was carried out to establish the extent of these epidemics, the damage caused, and the potential for forecasting their outbreaks in the future.

Thrips

Brachypterous adults of the cabbage thrips, *Thrips angusticeps*, emerging from their overwintering sites in the soil, attack the hypocotyls of young sugar-beet seedlings at the cotyledon stage . Most damage is caused just under the soil surface, and to the growing tips of the new true leaves. Their effects can be severe and are exacerbated by cold dry conditions; in 1996, over 500 hectares were redrilled as a result of their depredations.

Applications of insecticide sprays such as α-HCH, or the pyrethroids, cypermethrin, deltamethrin or lambda-cyhalothrin in two field trials in the worst affected areas (central Norfolk, and East Suffolk), failed to give adequate control, largely because the majority of the thrips were underground, and there was no penetration of these insecticides into the soil. However, anecdotal observations in other fields within the affected areas suggested that the seed treatment, imidacloprid ('Gaucho', Bayer), gave excellent control of these pests. Crops treated with carbamate granules, such as aldicarb ('Temik', Rhone-Poulenc) or carbofuran ('Yaltox' and others), were generally attacked less than

INSTITUTE OF ARABLE CROPS RESEARCH REPORT 1998

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FIG. 24 *Silver Y moth caterpillar*

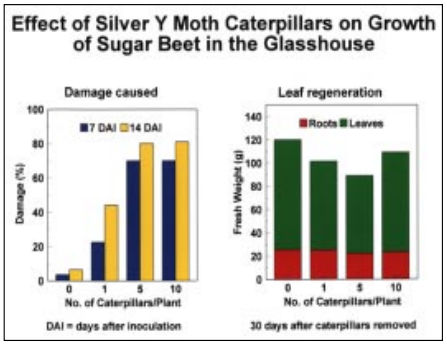

Fig. 25 *Regeneration of caterpillar-damaged beet*

untreated crops, but some still suffered severe damage perhaps because the systemic uptake of active ingredient from the granules was poor in the dry soil conditions.

Factors affecting the distribution of thrips were unclear, although there was a suggestion that damage was worse where sugar beet followed linseed in the rotation.

Caterpillars

Also in 1996, in June, reports of adults and eggs of the silver Y moth, *Autographa gamma*, were received, followed seven to ten days later by reports of many small caterpillars (Fig. 24). Adults of this species are migratory, originating each year from the Mediterranean region and North Africa. In 1996, there was an unprecedented migration, borne on warm southerly winds, from 5 June till the end of the month. The adults laid eggs in many broad-leaved crops, including potatoes, peas, beans, linseed and sugar beet. Three to four weeks after egg hatch substantial damage was recorded in many fields across the whole beet-growing area.

Well-timed applications of pyrethroid sprays (in late June) gave excellent control of these first generation caterpillars, but in many cases their presence was not noticed by farmers until large holes, caused by the feeding of fourth instar larvae, had appeared in the leaves. By the time sprays were applied in July the caterpillars had pupated, and were thus immune from the effects of the insecticides.

Many of the pupae of the first generation were parasitised by hymenopterous wasps, but those moths, which emerged from cocoons in late July and early August, did not lay their eggs in sugar beet. The female moths looking for oviposition sites may have been discouraged by the presence of other caterpillars or pupae on already-damaged plants.

No significant effects on yield were recorded at harvest. However, results from a glasshouse trial in which one, five or ten young caterpillars were placed on 8-10-leaf sugar-beet plants in pots showed that three caterpillars per plant appeared to be a suitable threshold for treatment. However, four weeks after removal of the caterpillars, which consumed up to 90% of the leaves, plants had compensated for the attack and there were no significant differences in fresh or dry weight (Fig. 25).

Spider mites

Infestations of two-spotted spider mites *Tetranychus urticae* were first recorded in early August in 1995 and 1996, causing yellowing symptoms on leaves similar to that caused by virus yellows . Damage was mostly confined to headlands, suggesting that spider mites had overwintered on weeds in field margins. The largest populations were seen in fields previously treated with pyrethroids to control caterpillars, suggesting that these insecticides might have upset the ecological balance between mites and their predators.

Several field trials to assess the efficacy of different acaricides against *T. urticae* produced no significant effects largely due to the variability in spider mite populations across the sites. Trial plots were inoculated with spider mites at Broom's Barn in July 1998 and populations were allowed to build up for six weeks before application of acaricides in late August. Tebufenpyrad ('Masai', Cyanamid), at 100 g a.i. ha^{-1}, significantly reduced spider mite numbers from 13 per leaf on untreated plants to less than two (1.8). Egg numbers were also decreased, but not significantly, from five to 0.9 per leaf. Of the other treatments, only bifenthrin ('Talstar', PBI Agrochemicals) gave significant control of active spider mites, but not of their eggs. However, there was no effect of the spider mites on yield in this trial.

The observations reported here show that, for some pests, especially long distance migrants, data on adequate control measures and thresholds for treatment have to be acquired opportunistically as and when the pests occur. Other pests, such as spider mites, can be cultured and this allows more controlled experimentation.

Alan Dewar - (IACR-Broom's Barn)

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RESEARCH ON THE EPIDEMIOLOGY AND MANAGEMENT OF TAKE-ALL DISEASE OF CEREALS

Take-all, normally caused by the fungus *Gaeumannomyces graminis* var. *tritici* (Fig. 26), is the most damaging root disease of cereals in the UK, where it was unusually prevalent in 1998, and in much of the rest of north-western Europe. Although it is soil-borne, the fungus does not survive well in the absence of its hosts (cereals, except oats, and a number of wild and cultivated grasses) and so the disease can be effectively controlled by rotating cereals with non-susceptible break crops. However, in the current economic climate, many growers find it more profitable to increase the areas of cereals under cultivation, relative to often less reliable break crops, even if this means growing a proportion of their cereal crops consecutively, thus risking a number of problems including damage by take-all. Most commentators agree that this trend will be reinforced if the provisions contained in the European Union's Agenda 2000 are implemented.

Options for managing take-all, apart from the use of appropriate rotations, are limited. Although cereal species differ in their susceptibility to the disease, there are no reliable differences between cultivars within a species. Take-all is also a difficult target for fungicides and none are currently available for commercial use. However, a number of companies are developing novel seed treatment compounds and some are expected to be on the market within a year or two.

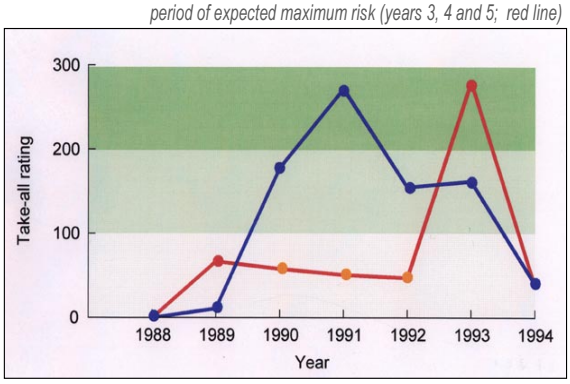
Take-all has long been the subject of investigation at IACR-Rothamsted where much of the emphasis has been on the kind of medium- to long-term field experimentation that is necessary to make progress in understanding the disease's complex behaviour. The selected examples which follow illustrate the current spectrum of research.

Occurrence of take-all in autumn-sown crops typically decreases with later sowing but we have shown that the benefits may be reduced by the presence of volunteers which help to maintain the fungus in the inter-crop period. Cereal volunteers are also important in the context of rotational set-aside, where their populations are typically very variable and, in contrast to most cereal crops, are usually destroyed before they reach maturity. To investigate the implications for a following crop, we established wheat 'volunteers' in mustard cover crops at densities ranging from 4 to 400 plants m^{-2}. Measurements of take-all in the following wheat showed that it had a close positive linear relationship with the square root of the population density of the 'volunteers' (corresponding to decreasing linear distances between individual 'volunteers'). There was also less disease where 'volunteers' had been destroyed in May rather than in August.

In consecutive cereal crops, take-all usually increases in severity over two to four years after which it typically becomes less severe. This phenomenon, known as 'take-all decline', is a form of natural biological control which, in continuous cereals, can provide modest but useful control of the disease. After the first demonstration of take-all decline, in experiments at Rothamsted, it was suggested that growers might be able to exploit this phenomenon, while avoiding the worst effects of the disease, by growing a relatively resistant cereal during the period of maximum risk and then changing to winter wheat (potentially the most profitable cereal) after decline had been established. Recent results from a long-term experiment testing different sequences of cereals have disproved this hypothesis. Take-all decline was shown to establish when each of the cereals tested was grown consecutively but followed a less severe peak in the case of a relatively resistant cereal such as triticale than it did with the much more susceptible wheat. However, interrupting a sequence of wheat crops with a less susceptible species (spring barley or triticale) during the period of expected maximum risk (i.e. from the third to the fifth crops) did not prevent severe disease when wheat cropping was resumed in the sixth year (Fig. 27). One of the worst sequences tested was alternating winter wheat and triticale. In this, take-all remained severe for much longer, before the establishment of take-all decline, than where winter wheat was grown without interruption. The results provide circumstantial evidence that the communities

Fig. 26 *A second wheat crop at Woburn Experimental Farm, showing effects of severe take-all*

Fig. 27 *Development of take-all epidemics in plots of continuous wheat (blue line) and in plots where spring barley was substituted for winter wheat during the period of expected maximum risk (years 3, 4 and 5; red line)*



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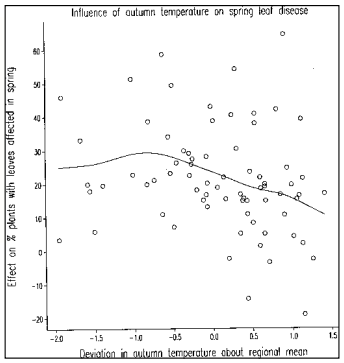


Fig. 28a *Influence of autumn temperatures on light leaf spot disease in spring*

of microorganisms that protect against take-all, and whose buildup may contribute to take-all decline, may be different in different cereals.

Communities of rhizosphere fungi are being studied in relation to stages in take-all epidemics and to disease risk in wheat, and in relation to take-all control by the new seed treatment fungicide fluquinconazole in wheat and barley. The take-all fungus itself is included in these studies. Of particular interest is a genotype (T2) identifiable by DNA probe, whose presence at more than 40% in populations sampled in wheat during the summer has, so far, always preceded the occurrence of patches of severe take-all in the following wheat crop. We have recently identified about 130 other species of sporulating micro-fungi in the rhizosphere, some of them rare or previously unrecorded either on wheat or in the UK. The current evidence is that although a few genera of rhizosphere fungi are often more frequent in continuous wheat than in wheat grown in rotations, there is some variation between sites and seasons. Their role, if any, in suppression of take-all has still to be confirmed.

Our research on new seed treatment fungicides for take-all control has concentrated on fluquinconazole, with support from AgrEvo. In three years of trials on wheat at Rothamsted and Woburn, decreases in disease severity on roots, assessed during grain filling, averaged 40% and increases in grain yield averaged 12%. Fluquinconazole acts against the take-all fungus but affects few other soil-inhabiting fungi. This suggests that seed treatment is unlikely to impair any natural suppression of take-all caused by fungi (except possibly those related to the take-all fungus, notably *Phialophora* spp.). A model system in which pot-grown wheat was dual-inoculated with the pathogen and one of several other fungi suggested that the efficacy of fluquinconazole may sometimes be enhanced by this specificity for the pathogen. Confirmation, or otherwise, of this is expected from detailed field experiments now in progress.

John Jenkyn - (IACR-Rothamsted)

FORECASTING SEVERITY OF LIGHT LEAF SPOT ON WINTER OILSEED RAPE

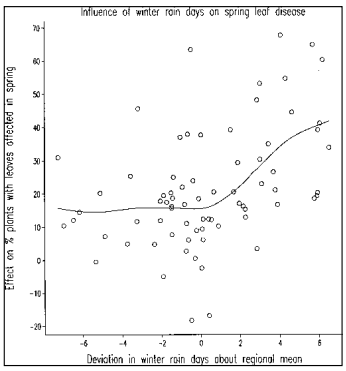
Light leaf spot, caused by *Pyrenopeziza brassicae*, is a serious disease of winter oilseed rape, which attacks leaves, stems and pods. Losses are greatest when severe attacks kill leaves, plants and even patches of crop during the winter. The disease has been estimated to have caused losses >£35M in the 1993/94 and 1994/95 seasons in the UK, despite the application of fungicides costing up to £9M[1]. However, ADAS/CSL survey data show that the severity of attacks differs greatly between seasons, between regions and between individual crops. Furthermore these data also indicate that fungicide use against the disease has not always been effective, often because fungicide timing has not been optimal. Thus, there is a need to develop a forecasting scheme to predict the development of severe epidemics at the optimum time for application of sprays to control them (in the autumn).

A provisional scheme for forecasting light leaf spot developed by IACR-Rothamsted in collaboration with ADAS, SAC and CSL, is being funded by H-GCA, MAFF and SOAEF. Investigation of patterns of light leaf spot disease by county indicated that England and Wales could be split up into seven regions, such that counties within regions showed similar patterns of disease across years. At the beginning of the growing season, in October, a regional risk index is produced which predicts the percentage of plants within the region which will show light leaf spot symptoms in March of the following year[2]. This risk index is derived from survey information for the previous July plus an adjustment for the long-term light leaf spot disease risk in each region. H-GCA issued these forecasts to the press in the autumns of 1996, 1997 and 1998, and the 1998 forecast was also made available on the World-Wide Web (URL http://www.res.bbsrc.ac.uk/molbio/LIs/). Thus, in October 1998, the forecast based on July 1998 survey data predicted that the proportion of crops with >25% of plants with light leaf spot in March 1999 would be greatest in northern England and smallest in East Anglia. Survey data in March 1997 and March 1998 confirmed that the October 1996 and October 1997 predictions followed the regional pattern of disease incidence correctly, although the observed percentage of plants with light leaf spot in a region was often less than predicted because many crops had been sprayed with fungicide.

Further work has shown that these regional risk indices can be updated during the growing season by incorporation of information on autumn temperature and winter rainfall in each region. Increased incidence of light leaf spot appears to be associated with regional autumn temperatures that are colder than the long-term average (Fig. 28a) and with an increased number of rain days in winter (Fig. 28b).

Regional risk indices can be supplemented by crop risk indices, which predict the risk to individual crops from information about the crop itself, such as sowing date and cultivar

Fig. 28b *Influence of number of winter rain days on light leaf spot disease in spring*



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susceptibility. Furthermore, a protocol has been developed for assessing the incidence of light leaf spot in crops considered to be at risk during the period between October and April, when sprays might be applied. Plants are sampled from the crop and the presence of the light leaf spot pathogen is confirmed by the development of white pustules (spore masses) on affected tissues (Fig. 29), after incubation in polyethylene bags at 10-15°C for four to seven days [3]. We have successfully used the polymerase chain reaction to diagnose *Pyrenopeziza brassicae* in infected, symptomless oilseed rape leaves [4]. However, since it would be difficult to develop this technique into a diagnostic kit for use by farmers or advisers, molecular methods are being used to identify specific proteins associated with infection by *P. brassicae* with a view to developing a simpler immuno-diagnostic test.

Use of this light leaf spot forecasting scheme has contributed to a change in the timing of many winter oilseed rape sprays from the spring to the autumn and to a decrease in light leaf spot incidence and associated yield losses in the 1995/1996, 1996/1997 and 1997/98 seasons. Field experiments validate the light leaf spot forecasting scheme and an interactive service is being developed on the World-Wide Web so that farmers can directly input information about their own crops. The accuracy of diagnosis of light leaf spot (for example in symptomless plants) is being improved and the epidemiology of the disease more clearly understood to ensure that the scheme is robust and reliable in different regions in different seasons and on different farms.

[1]Fitt, B. D. L. *et al.* (1997). *Aspects of Applied Biology* **48**, 135.

[2]Fitt, B. D. L. *et al.* (1996). In *Proceedings Brighton Crop Protection Conference- Pests and Diseases*, 239.

[3]Fitt, B. D. L. *et al.* (1998). *Annals of Applied Biology* **133**, 155.

[4]Foster, S. J. *et al.* (1998). *GCIRC Bulletin* **15**, 77.

Bruce Fitt and Sue Welham - (IACR-Rothamsted)

WILLOWS FOR SHORT-ROTATION COPPICE AND BIOREMEDIATION

Willows (*Salix* spp.), with over 300 species, show extensive variation in growth, physiology and biochemistry. This rich diversity underpins a versatility of uses from basket making, cricket bats and fibre, to short rotation coppice (SRC) and bioremediation. Research at IACR-Long Ashton (funded by BBSRC, EU, the Environment Agency, MAFF and industry partners) aims to underpin the use of willows as SRC and for bioremediation.

Much research focuses on SRC, which has environmental and socio-economic benefits (Box 1). SRC trees are planted at high densities, then harvested (coppiced) every three to five years. The cut stems are chipped and burnt for heat and to generate electricity. Increased bioenergy production is a priority in Europe, with a target equating to plantings on 8% of EU agricultural and forestry land (10 million hectares) by 2010. Improving biomass cultivars and pest and disease management are thus crucial research aims.

Genetic improvement of willow

The European Willow Breeding Programme was established at IACR-Long Ashton in January 1996, by a partnership of Long Ashton, Murray Carter and Svalöf-Weibull AB (Sweden), to produce high yielding, disease-and pest-

BOX1: BENEFITS OF SRC

Socio-economic benefits of short-rotation coppice to farmers are:

- income from a non-food crop
- guaranteed market for the product
- qualification for set-aside (though policy may change) or the Woodland Grant scheme
- enhancement of game cover on farm
- positive landscape options
- scope for amenity and recreational land use
- acceptable diversification for redundant arable land

Environmental benefits of short rotation coppice are:

- planting trees increases carbon fixation
- no net increase in atmospheric carbon dioxide
- reduces pesticide and fertiliser inputs
- wood chips produce alkaline or neutral flue gases when combusted
- the ashes are alkaline and can be used as soil conditioner
- 1 hectare of willow provides as much energy as 8 cubic metres of oil
- the energy balance is calculated at 30:1 positive ratio
- the ground is covered throughout the year enabling effective nutrient uptake
- significant increase in flora and fauna compared to conventional arable crops
- planting trees reduces soil erosion

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resistant varieties. Since then, 610 crosses have been made between representatives of 24 willow species from the National Willows Collection maintained at LARS. Over 25000 seedlings have been screened and the best 5-10% selected and advanced to large-scale field trials for further evaluation. Yield trials of the best 10-15 varieties will be established and from these one or two should be released by 2005.

The work on breeding is underpinned by molecular marker technologies. Many biomass willow clones are closely related genetically and can be difficult to distinguish using traditional morphological criteria. AFLP, the multi-locus fingerprinting technique developed by Keygene, is now used routinely for the identification of willow species, hybrids and varieties. This allows removal of duplicate individuals from the National Willow Collection and avoids repetitive crossing in the breeding programme. A preliminary genetic map from a population scored for rust resistance is being constructed using AFLPs and bulked segregant analysis is being applied in an attempt to isolate rust (*Melampsora* spp.) resistance markers.

Twenty highly polymorphic single-locus microsatellite markers in *Salix* have been developed and tested. These co-dominant markers can detect the presence of different alleles at one or more loci on homologous chromosomes and are amenable for use in databases. Further microsatellites are being characterised for identification and mapping of willow.

Fig. 29 *White pustales of* Pyrenopeziza brassicae *on leaves after incubation at 10-15°C*

Willow diseases and pests

Rust, caused by *Melampsora* spp., is the most damaging disease of SRC willows. Fourteen pathotypes have been identified, some infecting several varieties. New pathotypes, capable of causing severe disease on previously resistant willows, have evolved in recent years. In mono-cultures there is aggressive disease spread within a plantation but data from mixed plantings show consistent trends for delayed rust onset, reduced rate of rust spread, lower final rust severity and higher biomass yield when compared to monocultures of the same varieties. Rust pathotypes become more diverse as plantations mature, but there is no evidence yet for any major shift in pathogen composition, or the development of 'super races' as a result of deploying mixtures (Fig. 30).

Leaf-feeding chrysomelid beetles (*Phratora vulgatissima*, *P. vitellinae* and *Galerucella lineola)* are the most serious pests of SRC willows and can cause complete defoliation in extreme attacks. Beetle species differ in their feeding preferences, but most recommended SRC willow varieties are susceptible to one or more beetle species. Fewer beetles were shown to infest willows in mixed plantings compared to the same varieties grown as monocultures, with lower densities as the number of varieties in mixture increased from three to five and the structural composition became more random. Mixtures, therefore, also appear to have considerable potential value for limiting chrysomelid damage.

The population genetics of *P. vulgatissima* and *P. vitellinae* are being studied to provide further insights into their dynamics. A set of 13 microsatellites has been isolated and is being used to investigate population structure at several sites containing willow hosts for which the beetles have different feeding preferences, with sites separated by different distances.

Fig. 30 *Aerial view of willow trial showing monocultures of susceptible varieties defoliated by rust, but less severe damage to the same*

Potential of willow (*Salix viminalis*) to decrease unwanted nitrate from soil water in riparian buffer zones

SRC willow has great potential for nutrient retention and pollution amelioration in natural riparian

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buffer strips. Root tips of willow survive anaerobic waterlogged soil with sub-zero redox potentials for at least two weeks, which explains, in part, how the species can grow vigorously in riverside plantings. Such plantings have potential for decreasing the amount of nitrate entering the river in water draining from adjacent farmland. Laboratory experiments showed that willow can withstand atrazine pollution at levels exceeding those found in farm drains and is also capable of removing substantial amounts of nitrate (Fig. 31). Nitrogen in nitrate absorbed by the roots was transported to shoots in xylem sap as amino acids and glutamate with approximately 50% as nitrate. However, as external nitrate supply was raised, the proportion of nitrogen transported to the shoots as nitrate increased substantially. In solution culture tests, three categories of nitrate removal were identified: (i) nitrate needed to sustain root and shoot growth (growth nitrate) absorbed in association with increased activity in roots and shoots of the enzyme nitrate reductase; (ii) nitrate absorbed by roots and transferred to the shoots in amounts that exceed short term requirements for growth (luxury nitrate) but with no further increase in nitrate reductase activity; and (iii) nitrate transformed to other forms of nitrogen and lost from the system (discard nitrate). Discard nitrate was by far the largest category when external nitrate supplies exceeded 1mM and was probably lost in volatile forms, including ammonia and nitrous oxide. These possibilities are being investigated by laser analysis sensitive to one part per billion through the EU Life Sciences Trace Gas Facility at the University of Nijmegen, the Netherlands.

Fig. 31 *Rooted willow cuttings grown in solution culture and used to study the potential for removal of nitrate and atrazine from farmland run-off*

David Glen and Angela Karp - (IACR-Long Ashton)

DETECTING AND MEASURING NON-RANDOMNESS IN ECOLOGICAL COUNT DATA

In ecology it is often difficult to study the movement of individual animals directly, especially for small, numerous animals such as insects. Ecologists have, therefore, studied the pattern in two-dimensional space of individuals of a particular species to infer the underlying behavioural rules that govern their movement. It is being realised increasingly that the location of individuals in relation to one another may be critical for the outcome of ecological interactions. For example, the spatial heterogeneity resulting from non-random interactions between individuals, which arise from both inter- and intra-specific behaviour, is an important source of stability in ecological systems. Spatio-temporal dynamic ecological models such as cellular automata and metapopulation models increasingly use space explicitly, to locate and move individuals within a two-dimensional co-ordinate system. An ecological process that operates in a certain way at one scale may not operate in the same way, or at all, at a different scale.

Many ecological data, for example the number of weeds at specified locations, or of animals caught in traps, or of diseased plants within a quadrat, are recorded as integer counts (Fig. 32). Such data may be spatially referenced, when the position of each sample unit is recorded in two dimensions. Traditionally, the degree of heterogeneity, or non-randomness, in ecological systems was quantified by methods such as the relationship between the variance and mean of the counts. However, these operate solely on the list of counts and relate only to the numeric properties of the underlying frequency distribution; no use whatever is made of any additional spatial information in the sample. It is important to distinguish non-randomness in the form of numeric, statistical heterogeneity from truly spatial non-randomness. The former arises from skewness in the frequency distribution of counts and subsequent departures from a Poisson distribution; the latter arises from aggregation of those counts into clusters. Here, the term cluster is used to mean a region of either relatively large counts close to one another in two-dimensional space (i.e. a patch), or of relatively small counts (i.e. a gap).

Methods have been developed to analyse such data, using all of the spatial information available, in a way that is complementary to and independent of the purely numeric properties. The class of techniques is termed SADIE (Spatial Analysis by Distance IndicEs). At the heart of the techniques is the question: what would be the minimum effort that individuals would need to expend, to move from the observed arrangement to extreme arrangements, in which the individuals in the samples are either spaced as uniformly (regularly) or are as aggregated (crowded) as possible? In practice, this effort is equated with the total distance moved. Evidence shows that regularity is the most useful of the extreme arrangements. The minimum distance, *D*, required to move to complete regularity is provided by the Transportation Algorithm from the linear programming literature. This algorithm provides a unique solution based on notional 'flows' of individuals and fractions of individuals, from 'donor' sample units, with greater than average abundance, to 'receiver' units, with less than average abundance. The flows may be depicted graphically in a red-blue 'initial-and-final' plot that aids visual interpretation of the major spatial features of the data (Fig. 33). The spatial pattern is quantified by permuting the observed set of counts amongst the sample units. This provides data for the null hypothesis that the counts are arranged randomly with respect to one another, while maintaining precisely the numeric properties of the data, which may be investigated separately. In particular, division of the observed value of *D* by the mean value from several hundred such randomisations gives an index of aggregation, 1_a; values of $1_a = 1$ indicate randomly arranged counts, while $1_a > 1$ indicates aggregation of observed counts into clusters.

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Furthermore, comparison of the observed value of *D*, with the tails of the distribution of corresponding values from these permutations, leads to a usual formal randomisation test of the null hypothesis that the observed counts are arranged randomly. In this methodology no restriction is necessary for the arrangement of the sample units themselves; they may occupy any positions and do not need to be on a grid.

Developments of the SADIE methods have included: the analysis of mapped data[1], where the two-dimensional co-ordinates of each individual in the sample are known explicitly; an algorithm[2] that allows a set of given counts to be arranged over the given sample units to achieve any desired degree of spatial pattern or to provide another match for that pattern observed; allowance for edge effects[3]; and analysis of the spatial association[4] or dissociation between two populations measured simultaneously, or between the same population measured on two occasions, where the two populations are sampled at identical specified locations. Applications have included the development of efficient sampling schemes to monitor pest populations in the field; estimation of the point of introduction into the UK of an alien species; and a study of the latent period in virus transmission by aphids on lupins.

A more recent development recognises that a single index cannot encompass all the facets of spatial pattern in an arrangement. In particular, a single index does not provide evidence to infer whether a particular, large count represents some isolated, small sub-population, or alternatively, whether it is part of a patch to which neighbouring relatively large counts also contribute. Further, it does not answer whether, and to what degree, a group of nearby zero or small integer catches form a true gap that might indicate poorer than usual habitat, or whether their proximity to one another is no more than expected by chance, given the list of counts observed. The method underlying the new SADIE red-blue plot quantifies the degree to which the count for each sample unit contributes towards the overall degree of clustering, either as part of a patch or a gap(Fig. 34). It provides tests to detect the occurrence of clustering, and it facilitates a more formal definition of the size and dimension of clusters than that given previously.

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[2]Perry, J.N. (1996). *Computers & Electronics in Agriculture* **15**, 93.

[3]Perry, J.N. & Klukowski, Z. (1997). In *Proceedings VI Conference of the Biometric Society (Spanish Region), Córdoba, 21-24 September 1997*, 103.

[4]Perry, J.N. (1998). In *Population and Community Ecology for Insect Management and Conservation*. J. Baumgartner, P. Brandmayr & B.F.J. Manly (eds), Balkema, Rotterdam, 21.

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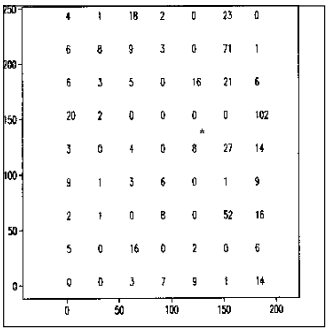

Fig. 32 *An example of a data set for spatial pattern analysis is counts of* Sitobion avenae *sampled over 63 sample units in a 9x7 rectangular grid at intervals of 30 m in a field of winter wheat. The centroid of the counts is denoted by an asterisk*

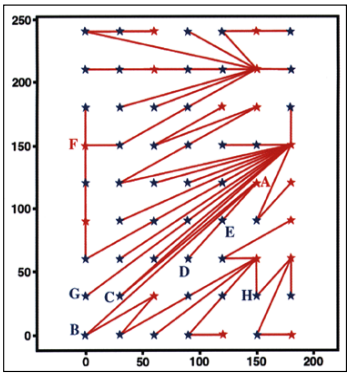

Fig. 33 *A SADIE 'initial-and-final' plot for the aphid data in Fig. 32. showing notional outflows, as red lines, originating from donor units with above-average counts, denoted by blue asterisks*

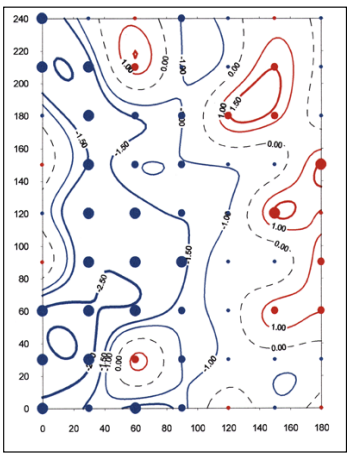

Fig. 34 *A map of clustering in the aphid data of Fig. 32. Patches are shown in red and gaps in blue. The size of the symbols,●, at grid points represent categories of clustering. Areas within contours with absolute value greater than 1.5 (thicker lines) indicate strong clustering in patches (red) or gaps (blue). Dashed lines are zero-value contours, where the interpolated count is close to the sample mean 8.8*