Ann. appl. Biol. (1988), **113**, 1–14
Printed in Great Britain

The effects of some commonly-used foliar fungicides on Collembola in winter barley: laboratory and field studies

BY G. K. FRAMPTON

Department of Biology, Building 44, The University, Southampton, Hampshire. SO9 5NH

(*Accepted 19 January 1988*)

SUMMARY

Four commonly-used cereal foliar fungicides were screened for their laboratory toxicity against the symphypleone collembolan, *Sminthurinus aureus*. A proportional hazards analysis of time-survival curves following the fungicide treatments showed that carbendazim, propiconazole, pyrazophos and triadimenol significantly increased the laboratory mortality of *S. aureus*. The organophosphorus fungicide pyrazophos caused high levels of mortality of *S. aureus* in the laboratory so a field evaluation of the effects of this fungicide on a wider range of Collembola was undertaken in winter barley.

Comparison of the effects of pyrazophos with those of the broad-spectrum insecticide dimethoate in the field revealed both compounds to have similar activity against some Collembola. Of the 11 species caught only the four symphypleone species exhibited these effects but the numbers of three symphypleone species were reduced to zero 4 wk after treatment with pyrazophos. The effects of pyrazophos and dimethoate were, however, not detectable in individual species after 11 wk.

INTRODUCTION

The ability of polyphagous predators to feed on crop pests, particularly cereal aphids, has formed the basis of extensive research (Sunderland, 1975; Vickerman & Sunderland, 1975; Sunderland & Vickerman, 1980; Chiverton, 1986, 1987). The importance of these predators in reducing cereal aphid numbers has been demonstrated using manipulative techniques (e.g. Edwards, Sunderland & George, 1979; Powell & Bardner, 1985; Chiverton, 1986). In addition to aphids, the diet of many polyphagous predators consists of other arthropods, including Collembola (Sunderland, 1975). In the linyphiid spiders, Collembola may constitute more than 60% of the diet (Sunderland, 1986). Collembola may also be the main dietary component for other cereal arthropods, for example the centipede *Lamycetes fulvicornis* Meinert (Sunderland, 1975). Surface-dwelling Collembola were investigated in this study in view of their importance in the diet of predators. The importance of polyphagous predators suggests that any direct toxicological or indirect (via the diet) side-effects of pesticides on them could influence their contribution to pest control. The widespread non-target effects of the broad-spectrum insecticide dimethoate (Vickerman & Sunderland, 1977) stimulated concern that other commonly-used pesticides might similarly be detrimental to beneficial arthropods in cereals.

Since their introduction in the early 1970s, the use of foliar fungicides has increased steadily. In 1974, *c.* 0·5 million spray ha were treated with foliar fungicides (*c.* 0·18 applications per crop) (Sly, 1981). The area of cereals treated in 1977 was almost double that in 1974, *c.* 0·9 million spray ha (*c.* 0·30 applications per crop), largely accounted for by the use of carbendazim (Steed, Sly, Tucker & Cutler, 1979; Sly, 1986). A further nine-fold increase led to

the treatment of *c.* 5 million spray ha (1·52 treatments per crop) in 1982, carbendazim and propiconazole being among the most widely-used products. Over 10 million spray ha are now treated annually with foliar fungicides (Hardy, 1986).

The insecticidal activity of pyrazophos has been demonstrated by Hassan & Franz (1973), Hassan (1974), van Zon & Wysoki (1978), Hassan (1979), Ledieu & Helyer (1983), Hassan *et al.* (1983), Parrella (1983), Sotherton & Moreby (1984) and Sotherton, Moreby & Langley (1987). Evidence that other fungicides adversely affect beneficial arthropods exists (e.g. Catling, 1969; Reyes & Stevenson, 1975; Vickerman, 1977; Vickerman & Sotherton, 1983; Wratten, Vickerman, Mead-Briggs & Jepson, 1988) but the effects of fungicides on Collembola are poorly known.

The aim of the present work is to investigate the possible side-effects of some commonly-used foliar fungicides on Collembola in cereals, in view of the potential importance of this group of arthropods and the increasing use of fungicides in the UK.

MATERIALS AND METHODS

Laboratory toxicology

Collembola cultures

A range of Collembola species were collected from winter wheat and winter barley using the D-Vac vacuum insect sampler (Dietrick, Schlinger & Garber, 1960). Collembola were kept in transparent Perspex boxes (54 cm × 60 cm × 90 cm) with sealable access windows and muslin-covered ventilation holes. Each contained a substrate of John Innes No. 2 compost sown with *Trifolium repens* (L.) as a source of food and cover. Fresh *T. repens* seedlings were transplanted from a glasshouse into the cultures when necessary. A relative humidity of 100% (measured using cobalt thiocyanate indicator paper with a 'Lovibond Comparator' humidity meter) was maintained by regular watering. The cultures were maintained at 16 °C (range 2 °C) and under a 16 h light cycle (light intensity 75 $\mu Em^{-2}s^{-1}$) for *c.* 9 months prior to testing.

Sminthurinus aureus (Lubbock) was considered the most suitable test species. It was most abundant in culture, less active and more easily handled than some of the larger arthropleone Collembola and was used for all laboratory experiments.

Choice of test substrate

A range of materials was tested for their suitability as substrates for the toxicological experiments. The high humidity requirement of Collembola (e.g. Davies, 1928, 1930) necessitated the use of substrates with good water-retention properties.

A single moistened filter paper disc gave a homogeneous mortality response but control mortality levels were still high (Fig. 1). In subsequent experiments, a wad of five filter paper discs was used. Longevity of Collembola in the controls was increased (Fig. 2) by moistening the lower four discs with 15 ml of distilled water and spraying the top disc with the appropriate test fungicide prior to placing it on the moistened discs.

Experimental enclosures

Experimental enclosures were designed to facilitate the observation of Collembola after trapping them on substrates previously treated with fungicide. The most suitable enclosures were inverted 9 cm-diameter Petri dishes, each with a muslin-covered ventilation hole in the lid (Petri dish base). Initially each enclosure contained a single 9 cm-diameter filter paper disc, but in subsequent experiments a wad of five filter paper discs was used in each enclosure, as described above. Movement of the small Collembola around the disc perimeter was

prevented by overlap of the enclosure lid with the disc. The lids were secured in place with transparent adhesive tape after introduction of the Collembola.

Choice of compounds

Compounds were chosen as representatives of the range of foliar fungicides commonly used on cereals in the UK. Pyrazophos ('Missile', Hoechst UK Ltd; 30% a.i.), an organophosphorus fungicide, was chosen in view of its known insecticidal activity. Carbendazim (Bavistin FL, BASF United Kingdom Ltd; 50% a.i.) was selected as a benzimidazole representative and the triazoles, propiconazole ('Tilt' 250EC, Ciba-Geigy Agrochemicals; 25% a.i.) and the more recently-introduced triadimenol (Bayfidan, Bayer UK Ltd; 25% a.i.), were chosen for comparison.

The test method

The fungicides were applied to the 9 cm-diameter filter paper substrate discs using a Potter Precision Spray Tower (Busvine, 1971). The Tower was calibrated to apply the compounds at rates equivalent to those recommended for use in the field (carbendazim, triadimenol and propiconazole at 0·5 litres/ha in 220 litres of water; pyrazophos at 2·0 litres/ha in 220 litres of water). Control substrates were sprayed with distilled water at a rate equivalent to 220 litres/ha.

Each fungicide was sprayed onto five filter paper substrate discs and five discs were used as controls. Each of the treated discs was transferred to a Petri dish. Initially, single treated discs were used but in subsequent experiments the treated disc was placed on four previously-moistened discs in each Petri dish, as described above.

Sminthurinus aureus was removed from the cultures using a modified aspirator (Southwood, 1978) and 10 adults (identified by their size) were placed at the centre of each treated substrate disc. Enclosures were then sealed immediately and maintained under controlled conditions. After initial checks to confirm that all 10 animals in each enclosure were alive, the numbers of living *S. aureus* in each enclosure were counted at intervals, initially hourly, following treatment.

Statistical analysis of time-survival curves

The relationships between percentage survival and time are shown in Figs 1 and 2. The survival curves were compared statistically using grouped data proportional hazards models similar to those described by Bartlett (1978).

Survival rates were analysed in terms of the number of animals at risk and the number which died during a given time interval for each treatment. The grouped data proportional hazards model was fitted using the computer package GLIM which fits generalised linear models.

Proportional hazards models. Models of the proportional hazards type quantify the difference between two survival curves by calculating a power to which the control or 'baseline' group (in this case those animals with the lowest mortality or highest survival) has to be raised in order to reach the mortality (or survival) function of the other group (the treated animals). The two groups of survival data are then compared using a parameter known as the relative risk which is a ratio of survival rates. The baseline group is assigned a relative risk (RR) of one. If, for example, the RR of another group under comparison is 50, then an animal in that group will stand a 50 times greater chance of dying in a given time interval than an animal in the baseline group.

The goodness-of-fit of each model was indicated by the deviance, a goodness-of-fit statistic (analogous to the residual sum of squares obtained from regression analysis) which in large samples has a Chi-squared distribution. The following three models were used to compare the effects of the fungicides on the survival of *Sminthurinus aureus* in the laboratory following treatment.

Time interval model. This model assumes that survival depends only on time (but not on treatments or replicates). Significantly large deviances were recorded in all comparisons indicating that the effect of time (grouped into intervals) alone did not adequately describe the survival patterns. One or more of the other explanatory factors were therefore added to the model.

Treatment effects model. This model added the treatment effects to the time interval model so that survival was assumed only to depend on time and treatments. The significance of the observed decrease in deviance was determined by use of Chi-squared tables with $n - 1$ degrees of freedom, where n treatments were under comparison. Significant decreases in deviance indicated a significant improvement in the goodness-of-fit by adding the treatment effects. Since treatments and controls were compared, significant decreases in deviance indicated a significant difference between the RR of the control and treated animals.

Replicates model. This model added the effects of replicates-nested-within-treatments to the treatment effects model so as to eliminate the risk that the effects of aberrant replicates might be interpreted as treatment effects. Significant decreases in the deviance (determined by use of Chi-squared tables with nr-2 degrees of freedom where n treatments each had r replicates) indicated further significant improvement in the goodness-of-fit and hence significant replicate effects. Where no such effects were shown, the treatment effect model was accepted. Where significant replicate heterogeneity was shown, the standard errors of the treatment RR estimates indicated whether significant RR differences still existed after accounting for the replicate heterogeneity.

Field evaluation of pyrazophos

The work was carried out in a 29·5 ha field of winter barley (cv. Halcyon), located *c.* 3·2 km north-west of Stockbridge, Hampshire (GR 330385, O.S. Sheet 185). The site was divided into nine plots, each of area *c.* 2·9 ha in order to accommodate a separate investigation into the effects of pyrazophos and dimethoate on mobile coleopteran and arachnid aphid predators. Although evidence is sparse, Collembola are generally considered rather immobile, particularly in comparison with some predatory insects so these large plots were considered acceptable for a Collembola study.

Trial design and fungicide application

The nine plots were arranged in a 3 × 3 Latin square design. There were three replicates of each of the pyrazophos, dimethoate and control (untreated) treatments. Other agrochemicals (Table 1) were applied to the entire field by the farmer. Pyrazophos and dimethoate ('Rogor E', FBC (Schering)) were applied to the appropriate plots on 2 May 1985 (G.S. 32; Zadoks, Chang & Konzak (1974)), pyrazophos at 600 g a.i./ha and dimethoate at 400 g a.i./ha, each in 224 litres/ha water at 30 p.s.i. from a tractor-mounted spray boom.

Sampling and identification of Collembola

A Dietrick Vacuum Insect Net (D-vac) was considered the most convenient method for sampling epigeal and hemiedaphic Collembola (G. K. Frampton, unpublished data) although it probably underestimated the numbers present (Sunderland *et al.*, 1986). A D-vac sample consisted of five randomly-placed 0·092 m^2 sub-samples, each involving an extraction period of 10 s which was considered sufficient to trap all the arthropods in the sampling area which

Table 1. *Treatments used at the study site in addition to the trial pesticides*

Date	Treatment
1984: 24 Sept. (barley sown)	methiocarb (Draza, 1·67 kg/ha)
26 Sept.	chlorpyrifos (Dursban, 1·5 l/ha)
31 Sept.	* chlorsulfuron + metsulfuron (Finesse, 100g/ha)
	* chlormequat (Cycocel, 0·75 l/ha)
1 Nov.	fenvalerate (Sumicidin, 0·2 l/ha
20 Nov.	tridemorph (Calixin, 0·75 l/ha
1985: 15 April	fluroxypyr (Starane, 1·0 l/ha; headlands only)
16 April	* benomyl (Benlate, 0·5 kg/ha)
	* chlormequat (Cycocel, 1·5 l/ha)
	* tridemorph (Calixin, 0·5 l/ha)
24 April	Cu solution (2·5 l/ha)
	Mg solution (2·5 l/ha)
3 May	sulphur (Thiovit, 0·53 kg/ha)
17 May	ethephon (Terpal, 2·0 l/ha)
	propiconazole (Tilt 250 EC, 0·5 l/ha)

* Tank mix.

could be caught by this method (Coombes, 1987). Each sample, therefore, represented an area of 0·46 m^2. Ten samples were taken along transects through the centre of each plot and each sample was transferred to 70% alcohol in the laboratory after storage at 4 °C for up to 24 h. Pre-treatment samples were taken on 30 April, 1985 and post-treatment samples on 10 May, 29 May, 13 June and 23 July, 1985.

Collembola species collected. Eleven species of Collembola were caught with the D-vac during the course of this investigation. These species, representing both suborders of Collembola, are listed in Table 2.

Extraction of Collembola. Organic material was extracted from inorganic soil material in the laboratory by repeated flotation with saturated sodium chloride solution (specific gravity 1·198). After two flotations a mean of 97% of the total collembola in the samples were extracted.

Identification of Collembola. Collembola were identified using the keys of Christiansen & Bellinger (1986), Fjellberg (1980), Gisin (1960, 1962, 1963, 1964*a*, *b*), Gough (1977), South (1961) and Stach (1947, 1956, 1960, 1963). High-power phase-contrast microscopy was necessary for the initial accurate identification of all the Collembola and subsequently for small and problematic specimens. Warmed lactophenol was used as a temporary mountant.

Taxonomic status of Sminthurinus aureus. The taxonomic status of *S. aureus* has been queried by some workers. Stach (1956) considered *S. aureus* to be synomymous with *S. elegans* and also with *S. quadrilineatus*, the difference being only in pigmentation. Fjellberg (1980), however, considered *S. aureus* and *S. elegans* to be different species. In order to maximise the information derived from this study, *S. aureus* and *S. elegans* will be regarded as distinct species here.

Statistical analysis of field trial data

Transformation of the data counts. Reasons for the transformation of data counts are summarised by Bartlett (1947) and Elliott (1977, p.30). Taylor (1961) found the power-law $s^2 = a\,m^b$, relating the variance, s^2 and mean, m of a sample to be an adequate description of the data counts obtained for a wide range of organisms. From the power-law, an appropriate variance-stabilising transformation can be obtained using the linear regression of $\log_{10}$ variance on $\log_{10}$ mean by raising data counts to a power of $1 - b/2$ (e.g. Healy & Taylor,

Table 2. *The significant treatment effects shown by analysis of variance on* $\log_{10}(n+1)$ *transformed counts*

Species	Date	Treatment effects
SUBORDER SYMPHYPLEONA:		
Sminthurus viridis (L.)	30 April	$P < 0{\cdot}01$
	10 May	$P < 0{\cdot}01$
	29 May	$P < 0{\cdot}05$
	13 June	$P < 0{\cdot}05$
Sminthurinus elegans (Fitch)	10 May	$P < 0{\cdot}01$
	29 May	$P < 0{\cdot}05$
S. aureus (Lubbock)	10 May	$P < 0{\cdot}05$
	29 May	$P < 0{\cdot}05$
Jeannenotia stachi (Jeannenot)	29 May	$P < 0{\cdot}05$
	13 June	$P < 0{\cdot}05$
SUBORDER ARTHROPLEONA:		
Isotoma viridis (Bourlet)	—	n.s.
I. notabilis (Schaffer)	—	n.s.
Isotomurus palustris (Muller)	—	n.s.
Lepidocyrtus cyaneus (Tullberg)	—	n.s.
Pseudosinella alba (Packard)	—	n.s.
P. decipiens (Denis)	—	n.s.
Hypogastrura denticulata (Bagnall)	—	n.s.
TOTAL SYMPHYPLEONA	10 May	$P < 0{\cdot}001$
	29 May	$P < 0{\cdot}01$
	13 June	$P < 0{\cdot}05$
	23 July	$P < 0{\cdot}01$
TOTAL ARTHROPLEONA	—	n.s.
TOTAL COLLEMBOLA	10 May	$P < 0{\cdot}05$
	29 May	$P < 0{\cdot}05$
	13 June	$P < 0{\cdot}05$

(n.s. denotes no significant treatment effect. Dates are given only where treatment effects were significant.)

1962), where a and b represent the intercept and regression coefficient respectively. Although the biological interpretation of the power-law is by no means clear, this has remained an effective method for transforming data counts prior to statistical analysis (Taylor, 1969; Taylor, Woiwod & Perry, 1980; Taylor & Woiwod, 1982).

A more convenient method of transforming data counts (J. Perry, personal communication) is to take $\log_{10}$ of the counts, x (or $\log_{10}(x+1)$ where zero counts are present). This method is advantageous in that calculations are straightforward and population events can be easily recognised, multiplicative density changes becoming additive after transformation. Both the power-law and $\log_{10}$ methods of transformation yielded similar results when compared and the $\log_{10}$ method was used subsequently (Table 2) in view of its simplicity. The adequacy of the transformation was confirmed by the independence of the sample variance and the sample mean following transformation of the data counts.

Pre-treatment spatial heterogeneity. Pre-treatment variability in the numbers of Collembola between the experimental plots was taken into account during the subsequent analysis by subtracting the $\log_{10}$-transformed mean pre-treatment counts from their corresponding $\log_{10}$-transformed mean post-treatment counts for each species and sampling date prior to analysis of variance. Temporal and spatial population density changes were assumed to be similar for all the experimental plots.

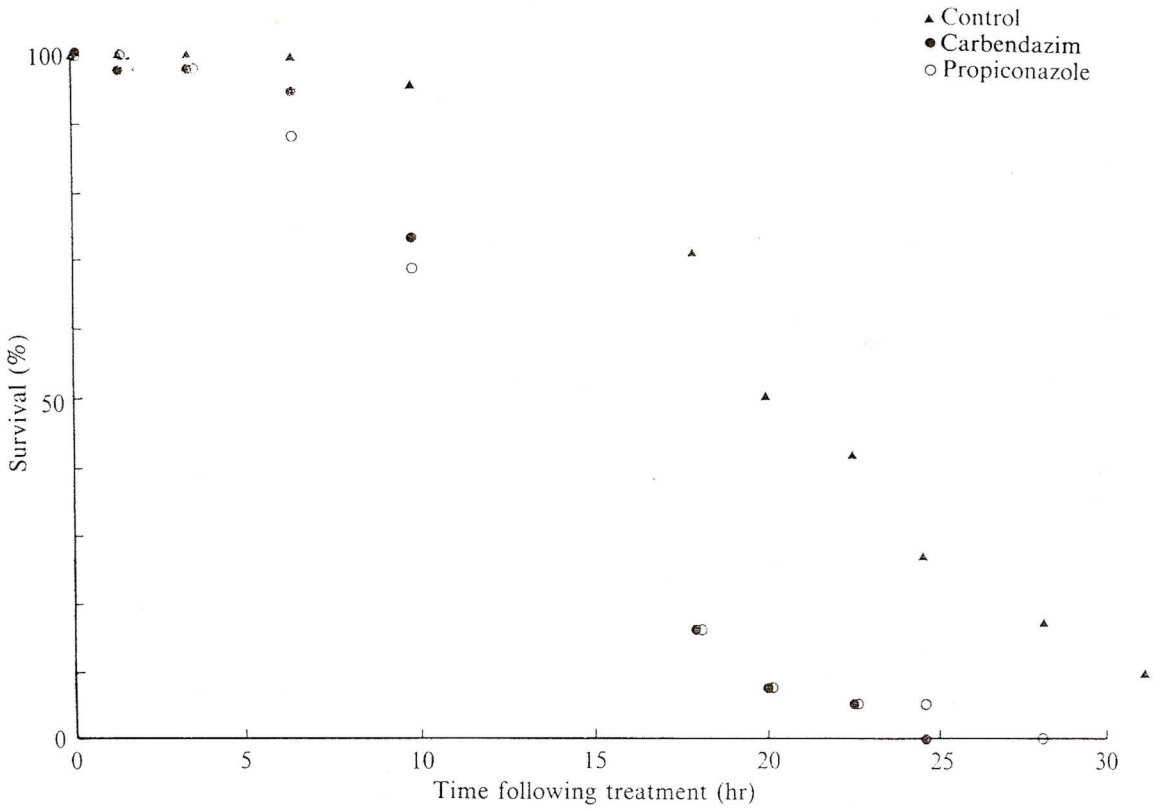

Fig. 1. The time-survival relationship for *Sminthurinus aureus* following laboratory treatment of the substrate with carbendazim and propiconazole.

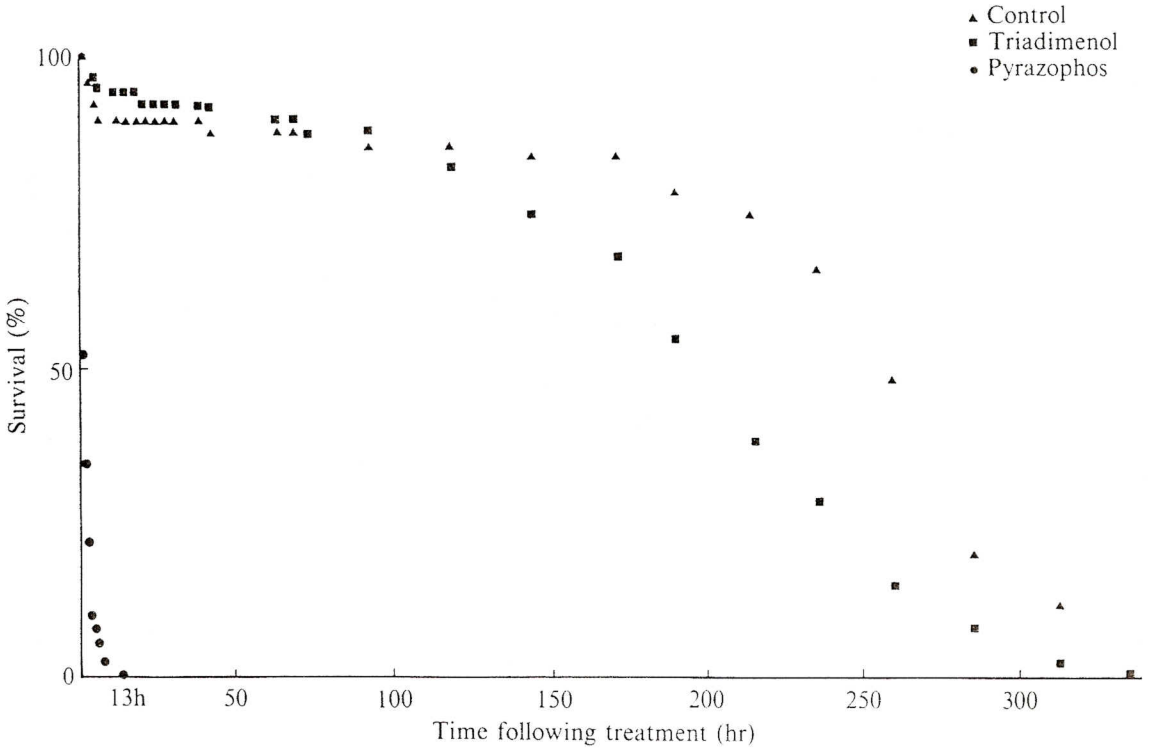

Fig. 2. The time-survival relationship for *Sminthurinus aureus* following laboratory treatment of the substrate with pyrazophos and triadimenol.

RESULTS

Laboratory toxicology

Interpretation of time-survival curves

The mean percentage survival of *Sminthurinus aureus* at different time intervals following treatment with the different fungicides is shown in Figs 1 and 2. In the first experiment (Fig. 1) the substrate was a single filter paper disc with limited water-retention qualities and the control mortality was high. In the second experiment (Fig. 2) the control mortality was substantially reduced using a wad of five moistened filter paper discs for each substrate. Despite the high control mortality in the first experiment, all fungicide treatments, carbendazim, propiconazole, pyrazophos and triadimenol significantly reduced the survival of *S. aureus* (Table 3). The organophosphorus fungicide pyrazophos was particularly potent (Fig. 2). There was 100% mortality in *S. aureus* 13 h after pyrazophos treatment, whilst control animals survived over 300 h.

Field evaluation of pyrazophos

The Latin square analysis of variance revealed significant treatment effects on four Collembola species. The post-treatment change in the numbers of each of these species relative to the pre-treatment numbers are shown in Fig. 3 and expressed as numbers per m^2 in Table 4.

Pre-treatment spatial heterogeneity

With the exception of *Sminthurus viridis*, no significant pre-treatment (30 April, 1985) spatial heterogeneity between plots was detected (Table 2). There were significant ($P < 0{\cdot}05$)

Table 3. *Statistics from a proportional hazards comparison of the survival curves of* Sminthurinus aureus *after different fungicide treatments*

Survival curve reference	Fungicide	Relative risk (with 95% CI range)	Decrease in deviance (χ^2; D.F. = 1)
Fig. 1	carbendazim	2·84 (1·13–7·19)	36·7
Fig. 1	propiconazole	4·99 (1·83–13·61)	33·6
Fig. 2	pyrazophos	98·79 (22·35–436·59)	110·0
Fig. 2	triadimenol	4·80 (1·85–12·43)	12·4

Table 4. *Species-date matrix showing the numbers of Collembola/m^2 in control (con), pyrazophos (pyr) and dimethoate (dim) treated plots*

	Pre-treatment 30 April			Post-treatment 10 May			29 May			13 June			23 July		
Species	con	pyr	dim	con	pyr	dim	con	pyr	dim	con	pyr	dim	con	pyr	dim
Sminthurus viridis	148	104	226	126	4	1	41	0	0	613	9	70	184	10	169
Sminthurinus elegans	1485	1432	1732	718	420	10	450	8	52	2498	1049	2549	10	11	9
S. aureus	27	19	27	17	11	0	28	0	0	24	2	17	0	0	0
Jeannenotia stachi	32	19	30	3	0	0	86	0	28	129	1	84	4	0	4
Total Symphypleona	1692	1574	2015	864	435	11	605	8	80	3264	1061	2720	198	21	183
Total Collembola	1714	1595	2051	881	445	23	682	21	132	3466	1087	2888	391	69	406

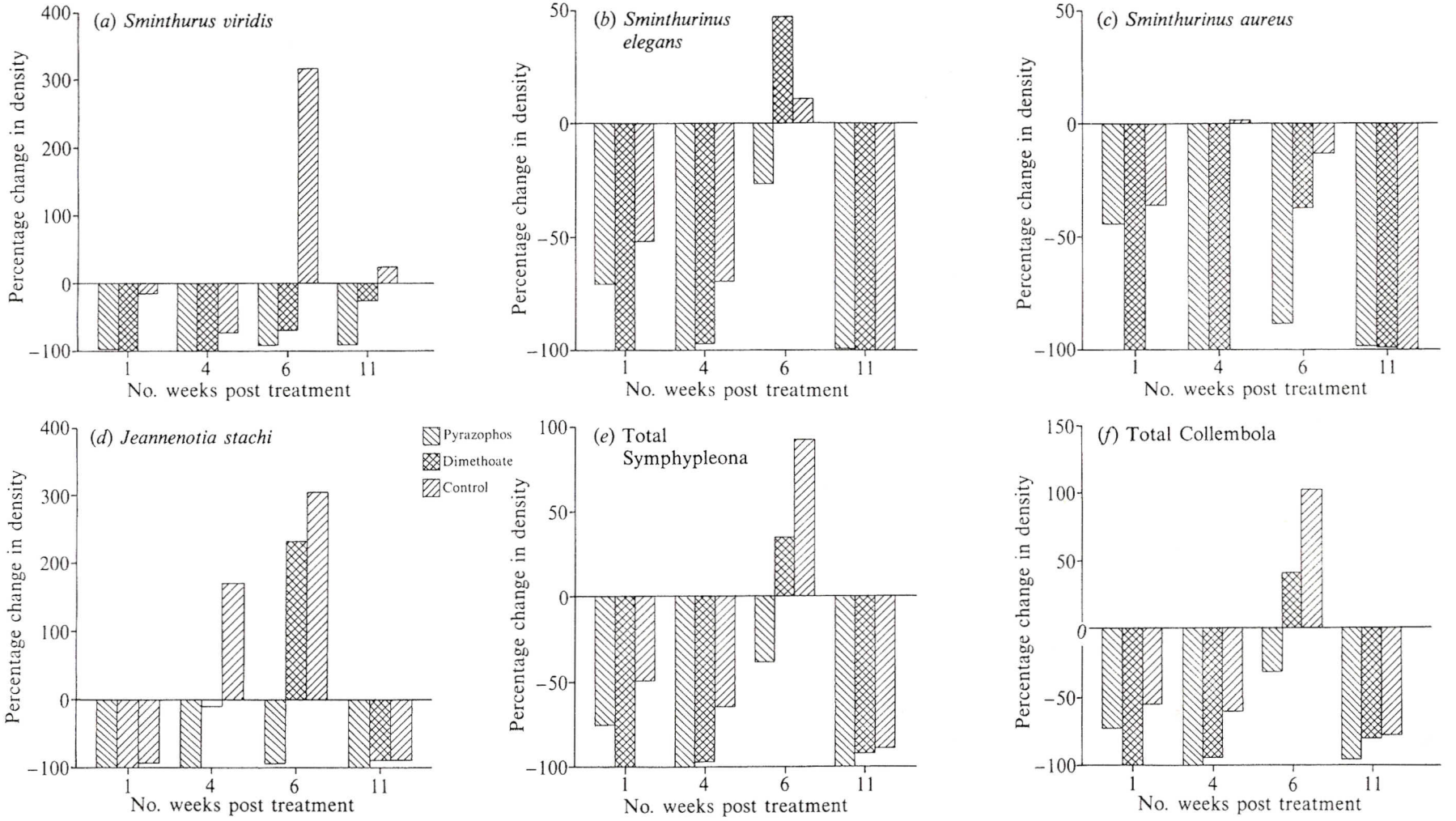

Fig. 3. Percentage changes in mean Collembola numbers (per m^2) on four post-treatment dates with respect to pre-treatment numbers. Positive changes show an increase in numbers with respect to pre-treatment numbers; negative changes indicate a decrease. With two exceptions, all error mean squares (E.M.S.) calculated from the analysis of variance on $\log_{10}$ counts were less than 20% of the $\log_{10}$ mean: E.M.S. values for 37 of the 45 histograms shown were less than 10% of the $\log_{10}$ mean. The exceptions were *S. viridis* on 10 May in control plots ($\log_{10}$ mean = 0·064, E.M.S. = 0·026) and *J. stachi* on 29 May in dimethoate plots ($\log_{10}$ mean = 0·201, E.M.S. = 0·050).

pre-treatment differences in the numbers of *S. viridis* between rows ($F = 119{\cdot}54$, D.F. = 2), between columns ($F = 71{\cdot}70$, D.F. = 2), and between treatment plots. Nevertheless, post-treatment data for *S. viridis* have been included since the change in numbers following treatment still paralleled those seen in the other species. Numbers of this species were always highest in the control plots following treatment (Fig. 3, Table 4).

Interpretation of the field trial results

Of the 11 species of Collembola found in the D-vac samples, the most abundant three, *Sminthurinus elegans, Sminthurus viridis* and *Jeannenotia stachi*, showed significant treatment effects (Tables 2 and 4). Although less abundant than some other species, *Sminthurinus aureus* was also significantly affected by the pyrazophos and dimethoate treatments (Fig. 3). In addition, Collembola in all treated plots were reduced in numbers with respect to controls 4 wk after treatment (Fig. 3).

In the control plots, all these species reached peak numbers on 13 June. The effects of dimethoate were more immediate than those of pyrazophos; 1 wk after treatment the lowest numbers of these species were found in dimethoate-treated plots. In contrast, 6 wk after treatment numbers were always least in the pyrazophos-treated plots. Similarly, the largest reductions in the numbers of Collembola were in dimethoate-treated plots 1 wk after treatment and in pyrazophos-treated plots 6 wk after treatment (Fig. 3). Three species, *Sminthurus viridis, Sminthurinus aureus* and *Jeannenotia stachi* were eliminated from the dimethoate plots 1 wk after treatment. These species were also absent from samples taken from pyrazophos-treated plots 4 wk post-treatment. *S. aureus* were also absent from samples taken from dimethoate-treated plots 4 wk after treatment (Table 4).

DISCUSSION

The results of the laboratory trials suggested that carbendazim, propiconazole, pyrazophos and triadimenol were toxic to Collembola. The effects of pyrazophos, the most potent compound, were also studied in winter barley while the other three compounds are currently being investigated in winter wheat.

The elimination of *Sminthurus viridis, Sminthurinus aureus* and *Jeannenotia stachi* from samples taken from dimethoate-treated plots 1 wk after treatment and their elimination from samples taken from pyrazophos-treated plots 4 wk after treatment provided evidence for the toxicity of both these organophosphorous compounds against Collembola. The reductions in the numbers of most species of Collembola after pyrazophos treatment were as large as those after dimethoate treatment (Fig. 3). This suggests that the insecticidal activity of pyrazophos against some Collembola is similar to that of dimethoate.

The insecticidal effects of dimethoate appeared to be more immediate than those of pyrazophos. The immediate effects of dimethoate are to be expected; this is a broad-spectrum insecticide. The time lag with pyrazophos (which was absent in laboratory studies) might be attributed to indirect effects through food – perhaps a reduction in the amount of saprophytic fungi, which most species of Collembola are thought to eat (e.g. Macnamara, 1924).

In contrast to the situation in dimethoate-treated and control plots, the numbers of Collembola in pyrazophos-treated plots never rose above the pre-treatment levels (Fig. 3). This is probably because the peak effect of pyrazophos coincided with the increase in Collembola numbers.

Of the 11 Collembola species caught in the D-vac, only the four symphypleone species exhibited significant reductions in numbers after pyrazophos and dimethoate treatments. In view of the suspected potency of these pesticides it is surprising that only four species were significantly reduced in numbers. Most of the seven arthropleone species were caught in

lower numbers than the symphypleone species; *Hypogastrura denticulata* and *Pseudosinella decipiens* were too rare in samples to warrant consideration here. Certainly it is possible that treatment effects could have been masked by inadequate data and high variability. The routine agrochemical applications might have influenced the variability in the data between dates but it is assumed any influence would be similar for all plots.

Methodological limitations and improvements

Sampling techniques

In this investigation, the D-vac vacuum insect sampling net was considered a suitable sampling method for hemiedaphic or epigeal Collembola, but this method would have inevitably underestimated collembolan densities (Sunderland *et al.*, 1986). The choice of sampling method had, however, to be a compromise between the labour cost (in terms of sampling and sorting effort) and the adequacy of the sampling method.

The problems of low arthropleone density and, to a certain extent, those of spatial heterogeneity might have been overcome by the additional use of pitfall traps. These provide an effective means of capturing some Collembola (Joosse, 1965). Pitfall traps were, in fact, used at the study site by an independent investigation and the collembolan contents of these will be presented elsewhere.

The experimental design

In view of the limited land availability, with independent studies also being undertaken at the study site, an unavoidable limitation of this study was the use of nine large plots allowing three replicates per treatment. Ideally, a larger number of smaller plots would have been better to allow for more replicates and therefore provide better information on and control of the spatial variability in the data. However, the risk of immigration to and emigration from treated plots must be considered before plot size is chosen; that treatment effects in individual species could not be detected 11 wk after treatment does not necessarily imply degradation or dilution of the pesticide. For small plots, immigration and emigration would require monitoring or checking by the use of exclusion barriers. Such a technique is currently in use in the investigation of the effects of carbendazim, propiconazole and triadimenol on Collembola in winter wheat.

CONCLUSIONS

The four foliar fungicides, carbendazim, propiconazole, pyrazophos and triadimenol killed *Sminthurinus aureus* in the laboratory. Pyrazophos, known to have an insecticidal activity (Martin & Worthing, 1976; Sotherton & Moreby, 1984; Sotherton *et al.*, 1987) appeared to exhibit a level of activity similar to that of the broad-spectrum insecticide dimethoate with the capability of reducing field densities of some species of Collembola by up to 100% with respect to controls. Of the 11 species of Collembola caught, however, the seven arthropleone species were apparently unaffected by pyrazophos and dimethoate although effects could have been masked by low densities of individuals and high spatial heterogeneity.

Given the potential importance of Collembola in the cereal ecosystem and the steady increase in the use of foliar fungicides in the UK, further research into non-target effects of fungicides is evidently needed.

ACKNOWLEDGEMENTS

The work presented here forms part of a studentship funded by the Ministry of Agriculture, Fisheries and Food. This work was supervised by Dr G. P. Vickerman and Dr S. D. Wratten,

to whom thanks are due for their help. The author is grateful to Harold Gough of I.C.I. Plant Protection Division and Dr Mark Wetton of the British Museum for their assistance in the identification of Collembola and to Mr Joe Perry of Rothamsted Experimental Station for statistical advice.

Thanks are also due to the staff of the Leckford Estate, especially Ben Gibbons, without whom the field trials would have been restricted. The assistance of Hoechst (U.K.) Ltd, especially Dr R. T. Hewson, is also gratefully acknowledged for allowing participation in the 1985 pyrazophos field trial.

REFERENCES

BARTLETT, M. S. (1947). The use of transformations. *Biometrics* **3**, 39–52.

BARTLETT, N. R. (1978). A survival model for a wood preservative trial. *Biometrics* **34**, 673–679.

BUSVINE, J. R. (1971). *A Critical Review of the Techniques for Testing Insecticides*, 2nd ed. Commonwealth Agricultural Bureaux. 345 pp.

CATLING, W. S. (1969). Benomyl – a broad-spectrum fungicide. *Proceedings 5th British Insecticide and Fungicide Conference* **2**, 298–309.

CHIVERTON, P. A. (1986). Predator density manipulation and its effects on populations of *Rhopalosiphum padi* (Hom.: Aphididae) in spring barley. *Annals of Applied Biology* **109**, 49–60.

CHIVERTON, P. A. (1987). Predation of *Rhopalosiphum padi* (Homoptera : Aphididae) by polyphagous predatory arthropods during the aphids' pre-peak period in spring barley. *Annals of Applied Biology* **111**, 257–269.

CHRISTIANSEN, K. & BELLINGER, P. F. (1986). Collembola of North America north of the Rio Grande (Part I). Grinnel College, Grinnel, Iowa 50112.

COOMBES, D. S. (1987). *Factors limiting the effectiveness of* Demetrias atricapillus *(L.) (Coleoptera: Carabidae) as a predator of cereal aphids.* Unpublished Ph.D. Thesis. University of Southampton.

DAVIES, W. M. (1928). The effect of variation in relative humidity on certain species of Collembola. *British Journal of Experimental Biology* **6**, 79–86.

DAVIES, W. M. (1930). The influence of humidity on Collembola. *Report on the Agricultural Meteorologial Conference. Ministry of Agriculture & Fisheries, London*, pp. 46–51.

DIETRICK, E. J., SCHLINGER, E. I. & GARBER, M. J. (1960). Vacuum cleaner principle applied in sampling insect populations in alfalfa fields by new machine method. *California Agriculture*, January 1960, pp. 9–11.

EDWARDS, C. A., SUNDERLAND, K. D. & GEORGE, K. S. (1979). Studies on polyphagous predators of cereal aphids. *Journal of Applied Ecology* **16**, 811–823.

ELLIOTT, J. M. (1977). *Some Methods for the Statistical Analysis of Samples of Benthic Invertebrates*, 2nd ed. Freshwater Biological Association Publication No. 25.

FJELLBERG, A. (1980). *Identification keys to Norwegian Collembola.* The Norwegian Entomological Society (Norsk Entomologisk Forening), 152 pp.

GISIN, H. (1960). *Collembolenfauna Europas.* Muséum d'Histoire naturelle, Geneva, 312 pp.

GISIN, H. (1962). Sur la faune européene des Collemboles IV. *Revue Suisse de Zoologie* **69**, 1–23.

GISIN, H. (1963). Collemboles d'Europe V. *Révue Suisse de Zoologie* **70**, 77–101.

GISIN, H. (1964*a*). Collemboles d'Europe VI. *Révue Suisse de Zoologie* **71**, 383–400.

GISIN, H. (1964*b*). Collemboles d'Europe VII. *Révue Suisse de Zoologie* **71**, 649–678.

GOUGH, H. J. (1977). A key for the identification of the families of Collembola recorded from the British Isles. *Entomologists' Monthly Magazine* **113**, 193–197.

HARDY, A. R. (1986). The Boxworth Project – A progress report. *Proceedings 1986 British Crop Protection Conference – Pests and Diseases* **3**, 1215–1225.

HASSAN, S. A. (1974). A method for the investigation of the action of pesticides on egg parasites of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) – results of a series of experiments with fungicides. *Zeitschrift für Angewandte Entomologie* **76**, 120–134.

HASSAN, S. A. (1979). Integrated control on glasshouse vegetable crops. In *Agriculture and Forestry*, pp. 485–486. Proceedings International Symposium of the International Organisation for Biological Control/WPRS: Integrated Control, Vienna.

HASSAN, S. A. & FRANZ, J. M. (1973). Die Wirkung von Pflanzenschutzmitteln auf Eiparasiten der Gattung Trichogramma-Prüfmethoden und Ergebnisse. *Proceedings 39th German Plant Protection Conference of the State Institute for Agriculture and Forestry*, Stuttgart, pp. 196–197.

HASSAN, S. A., BIGLER, F., BOGENSCHUTZ, H., BROWN, J. U., FIRTH, S. I., HUANG, P., LEDIEU, M. S., NATON, E., OOMEN, P. A., OVERMEER, W. P. J., RIECKMANN, W., SAMSOE-PETERSEN, L., VIGGIANI, G. & ZON, A. Q. VAN (1983). Results of the Second Joint Pesticide Testing Programme by the IOBC/WPRS-Working Group 'Pesticides and Beneficial Arthropods'. *Zeitschrift für Angewandte Entomologie* **95**, 151–158.

HEALY, M. J. R. & TAYLOR, L. R. (1962). Tables for power law transformations. *Biometrika* **49**, 557–559.

JOOSSE, E. N. G. (1965). Pitfall-trapping as a method for studying surface dwelling Collembola. *Zeitschrift für Morphologie und Ökologie der Tiere* **55**, 587–596.

LEDIEU, M. S. & HELYER, N. L. (1983). Pyrazophos: a fungicide with insecticidal properties including activity against chrysanthemum leaf miner (*Phytomyza syngenesiae*) (Agromyzidae). *Annals of Applied Biology* **102**, 275–279.

MACNAMARA, C. (1924). The food of Collembola. *Canadian Entomologist* **56**, 99–105.

MARTIN, H. & WORTHING, C. R. (1976). *Insecticide and fungicide handbook*, 5th ed. British Crop Protection Council. Blackwell, London. 427 pp.

PARRELLA, M. P. (1983). Evaluations of selected insecticides for control of permethrin-resistant *Liriomyza trifolii* (Diptera: Agromyzidae) on Chrysanthemum. *Journal of Economic Entomology* **76**, 1460–1464.

POWELL, W. & BARDNER, R. (1985). Effects of polyethylene barriers on the numbers of epigeal predators caught in pitfall traps in winter wheat with and without soil-surface treatments of fonofos. Working Group 'Integrated Control of Cereal Pests', *International Organisation for Biological Control WPRS Bulletin 1985/VIII/3*, pp. 136–138.

REYES, A. A. & STEVENSON, A. B. (1975). Toxicity of benomyl to the cabbage maggot, *Hylemya brassicae* (Diptera: Anthomyiidae) in greenhouse tests. *Canadian Entomologist* **107**, 685–687.

SLY, J. M. A. (1981). Review of usage of pesticides in agriculture, horticulture and forestry in England and Wales 1975–1979. *Pesticide Usage Survey Report 23*, M.A.F.F., London. 94 pp.

SLY, J. M. A. (1986). Arable farm crops and grass 1982. Survey Report 35. *Reference book 535*, M.A.F.F., London, pp. 44–67.

SOTHERTON, N. W. & MOREBY, S. J. (1984). Contact toxicity of some foliar fungicide sprays to three species of polyphagous predators found in cereal fields. *Tests of Agrochemicals and Cultivars, No. 5* (*Annals of Applied Biology* **104**, *Supplement*), pp. 16–17.

SOTHERTON, N. W., MOREBY, S. J. & LANGLEY, M. G. (1987). The effects of the foliar fungicide pyrazophos on beneficial arthropods in barley fields. *Annals of Applied Biology* **111**, 75–87.

SOUTH, A. (1961). The taxonomy of the British species of *Entomobrya* (Collembola). *Transactions of the Royal Entomological Society, London* **113**, 387–416.

SOUTHWOOD, T. R. E. (1978). *Ecological methods with special reference to the study of insect populations*, 2nd ed. Chapman and Hall, London. 524 pp.

STACH, J. (1947). The apterygotan fauna of Poland in relation to the world fauna of this group of insects: Isotomidae. *Nakl. Polskiej Akademii Umiejétnosci*, Krakow. 488 pp.

STACH, J. (1956). The apterygotan fauna of Poland in relation to the world fauna of this group of insects: Sminthuridae. *Polska akademia nauk Instytut zoologiczny*. 287 pp.

STACH, J. (1960). The apterygotan fauna of Poland in relation to the world fauna of this group of insects: Orchesellini. *Polska akademia nauk Instytut zoologiczny*. 151 pp.

STACH, J. (1963). The apterygotan fauna of Poland in relation to the world fauna of this group of Insects: Entomobryini. *Polska akademia nauk Instytut zoologiczny*. 126 pp.

STEED, J. M., SLY, J. M. A., TUCKER, G. G. & CUTLER, J. R. (1979). Arable farm crops 1977. *Survey Report* **18**, pp. 17–164. M.A.F.F., London.

SUNDERLAND, K. D. (1975). The diet of some predatory arthropods in cereal crops. *Journal of Applied Ecology* **12**, 507–515.

SUNDERLAND, K. D. (1986). Spiders and cereal aphids in Europe. *International Organisation for Biological Control WPRS Bulletin 1987/X/1*, pp. 82–102.

SUNDERLAND, K. D., & VICKERMAN, G. P. (1980). Aphid feeding by some polyphagous predators in relation to aphid density in cereal fields. *Journal of Applied Ecology* **17**, 389–396.

SUNDERLAND, K. D., HAWKES, C., STEVENSON, J. H., McBRIDE, T., SMART, L. E., SOPP, P. I., POWELL, W., CHAMBERS, R. J. & CARTER, O. C. R. (1986). Accurate estimation of invertebrate density in cereals. *International Organisation for Biological Control WPRS Bulletin 1987/X/1*, pp. 71–81.

TAYLOR, L. R. (1961). Aggregation, variance and the mean. *Nature, London* **189**, 732–735.

TAYLOR, L. R. (1969). Aggregation as a species characteristic. *Statistical Ecology. Proceedings International Symposium New Haven Connecticut* **1**, pp. 357–377.

TAYLOR, L. R., WOIWOD, I. P. & PERRY, J. N. (1980). Variance and the large scale spatial stability of aphids, moths and birds. *Journal of Animal Ecology* **49**, 831–854.

TAYLOR, L. R. & WOIWOD, I. P. (1982). Comparative synoptic dynamics. I. Relationship between inter- and intra-specific spatial and temporal variance/mean population parameters. *Journal of Animal Ecology* **51**, 879–906.

VICKERMAN, G. P. (1977). The effects of foliar fungicides on some insect pests of cereals. *Proceedings 1977 British Crop Protection Conference – Pests and Diseases*) **1**, 121–128.

VICKERMAN, G. P. & SUNDERLAND, K. D. (1975). Arthropods in cereal crops: Nocturnal activity, vertical distribution and aphid predation. *Journal of Applied Ecology* **12**, 755–766.

VICKERMAN, G. P. & SUNDERLAND, K. D. (1977). Some effects of dimethoate on arthropods in winter wheat. *Journal of Applied Ecology* **14**, 767–777.

VICKERMAN, G. P. & SOTHERTON, N. W. (1983). Effects of some foliar fungicides on the chrysomelid beetle *Gastrophysa polygoni* (L.) *Pesticide Science* **14**, 405–411.

WRATTEN, S. D., VICKERMAN, G. P., MEAD-BRIGGS, M. & JEPSON, P. C. (1988). Effects of the fungicide pyrazophos on predatory and parasitic insects in winter barley. *Proceedings BCPC Symposium on Field Methods for the study of environmental effects of pesticides*. Cambridge, March 1988 (in press).

ZADOKS, J. C., CHANG, T. T. & KONZAK, C. R. (1974). A decimal code for the growth stages of cereals. *Weed Research* **14**, 415–421.

ZON, A. Q. VAN & WYSOKI, M. (1978). The effects of some fungicides on *Phytoseiulus persimilis* (Acarina: Phytoseiidae). *Entomophaga* **23**, 371–378.

(*Received 12 August 1987*)