Spatial variation in non-target effects of the insecticides chlorpyrifos, cypermethrin and pirimicarb on Collembola in winter wheat

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Abstract: Contiguous winter wheat fields of similar cropping history and soil type were used in a study of the responses of Collembola to summer sprays of cypermethrin and pirimicarb in southern England. Chlorpyrifos was included in the study as a toxic standard. Epigeic arthropods were captured by suction sampling and crop-inhabiting species obtained by dissecting wheat ears. Eight genera of Collembola responded significantly to the insecticide treatments. Collembolan abundance decreased after chlorpyrifos was applied but increased after use of cypermethrin. Negative effects of cypermethrin and pirimicarb on Collembola were not detected in this study. Effects of chlorpyrifos varied spatially as a result of faunal heterogeneity among the fields, despite apparent homogeneity of the site. Some species known to be susceptible to chlorpyrifos were absent from one or more of the fields. The implications of these findings for the interpretation of non-target pesticide effects and the potential use of Collembola as bioindicators in field studies with pesticides are discussed.

Keywords: insecticides; chlorpyrifos; cypermethrin; pirimicarb; Collembola; side-effects; bioindicators

1 INTRODUCTION

Collembola (springtails) are among the most abundant of the non-target arthropods to be found in temperate arable farmland,[1] where they are preyed upon both by stenophagous[2,3] and polyphagous predatory arthropods.[4] At certain times of the year Collembola may provide a large proportion of the diet of some species of Araneae (spiders) and Coleoptera (beetles)[5–8] including species ranked as potentially important predators of cereal pests in Europe.[9,10] The occurrence of Collembola in arable fields early in the season could be significant in sustaining such predator populations and enhancing subsequent control of pests.[11] However, the ecology of many species is relatively poorly understood despite their abundance and widespread distribution,[12] a situation which appears to be typical of most farmland arthropods not deemed to be of immediate economic significance.[13] With the possible exception of the lucerne-flea *Sminthurus viridis* L, which occasionally reaches pest status locally in southern Europe,[14] or edaphic Onychiuridae in sugar beet,[15] European Collembola appear overall to be beneficial on account of their importance as prey and participation in nutrient cycling.[16]

Numerous field studies have investigated the responses of predatory arthropods to the carbamate aphicide pirimicarb[17–25] and to synthetic pyrethroid insecticides such as cypermethrin,[17,20,23,26–31] deltamethrin,[21,22,28,29,32–36] fenvalerate[37–40] and lambda-cyhalothrin.[30,39,41,42] Cypermethrin is currently the most widely used insecticide in arable crops in Britain, and in 1996 was applied to 54% of the arable area grown (60% of the area of wheat), whilst pirimicarb was applied to 6% of the arable area.[43] Despite the widespread use of these insecticides and extensive studies of their effects on predators, very little is known about effects of these insecticides on Collembola in arable fields.[44]

Semi-field methods such as mesocosms[45] or bioassays[46] have been used to investigate effects of pesticides on Collembola, but these approaches have so far involved a relatively small number of species. To date, no studies have been designed specifically to investigate effects of pirimicarb or cypermethrin on field-resident assemblages of Collembola in arable crops. Some observations on effects of these insecticides have been made during studies which focused on other non-target arthropod groups, but results have largely been equivocal, either because Collembola were not the taxon of primary interest,[23,27] their responses to pesticides were not presented in detail,[47] or because effects of individual insecticides were confounded with other variables.[48] Use of cyperme-

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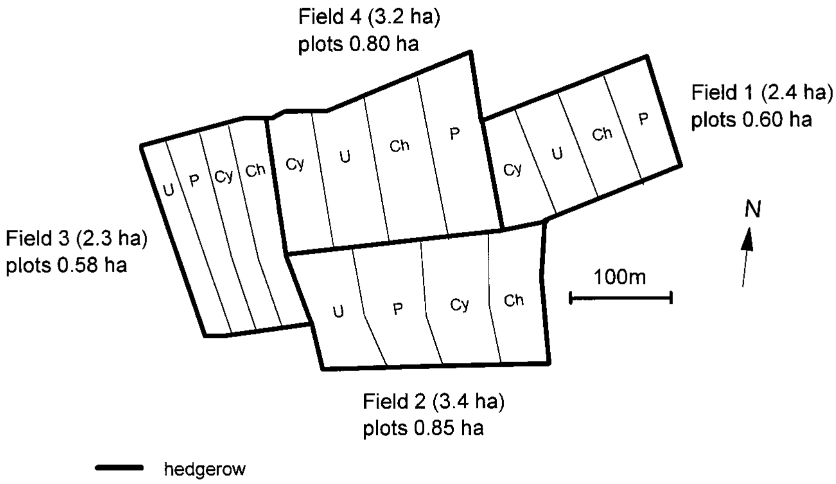

Figure 1. Layout of unsprayed (U), chlorpyrifos (Ch), cypermethrin (Cy) and pirimicarb (P) treatments in four contiguous fields of winter wheat.

Table 1. Soil characteristics, October 1994

Property[a]	*Field 1*	*Field 2*	*Field 3*	*Field 4*
pH	6.9	6.5	6.5	6.3
Organic matter (%)	8.8	6.8	6.2	4.4
Coarse sand (%)	26.9	21.9	33.3	34.7
Fine sand (%)	34.4	38.0	31.5	29.1
Silt (%)	15.9	21.0	15.6	16.1
Clay (%)	15.5	12.8	9.8	11.7
Saturation capacity (%)	88.9	76.4	78.5	77.4
P (mg litre^{-1})	70	31	23	31
K (mg litre^{-1})	272	216	102	192
Mg (mg litre^{-1})	101	107	48	107

[a] Soil analysis: estimates of mean organic matter contents (loss-on-ignition), particle composition (Bouyoucos hydrometer) and saturation capacity followed the methods given by Allen.[52] pH and nutrient status were obtained from farm records.

thrin in forestry was found to increase pitfall trap catches of Collembola after some sprays, possibly because predatory Carabidae, Staphylinidae and Linyphiidae were negatively affected.[31] Studies with other synthetic pyrethroids have shown mixed effects on Collembola: in barley, fenvalerate, but not permethrin, decreased pitfall catches of Collembola in small ($10\,\mathrm{m} \times 10\,\mathrm{m}$) plots,[40] whilst in pine litter Collembola numbers were unaffected by permethrin applied at a rate higher than that recommended for agricultural use.[49] Effects of lambda-cyhalothrin on Collembola varied spatially in hops, with increased catches after spraying in one hop garden but decreased catches in another.[42] Attempts to control *Sminthurus viridis* in lucerne in Australia showed that fenvalerate was ineffective[50] whilst in eastern Spain catches of this species increased on some occasions after spraying with lambda-cyhalothrin.[14] Pitfall catches of Collembola in a sugar beet crop in England were unaffected by a granular application of tefluthrin.[51] Relatively few data exist on inter-specific variation in responses of Collembola to synthetic pyrethroids and pirimicarb[46] and in only one of the field studies mentioned above[42] were responses of individual species considered.

The present study was carried out in 1994 to investigate effects on Collembola of chlorpyrifos, cypermethrin and pirimicarb in winter wheat. This paper presents responses of field-inhabiting species to summer applications of these insecticides in four contiguous fields. The findings of this work are compared with those of a previously published study[46] which used in-situ field bioassays in one of the study fields to expose selected collembolan test species to residues of the insecticides.

2 MATERIALS AND METHODS

2.1 Experimental site

The experimental site was located at Mereworth (TQ 660535) in south-east England (51° 16′ N, 0° 23′ E) and comprised four contiguous fields under winter wheat (*Triticum aestivum* L cv Hereward, drilled 14 October 1993) (Fig 1). These fields were chosen for the study because they had similar soil properties (sandy clay loam: Table 1) and had been farmed previously as a single unit, having received similar cropping (and pesticide and fertiliser inputs) for at least six years prior to the study (Table 2). The fields were mostly enclosed by, and shared, hedgerows of similar age dominated by hawthorn (*Crataegus monogyna* Jacq). Fertilisers and agrochemical applications other than the trial insecticides (Table 3) were applied to all study fields together as deemed necessary by the farm manager.

Table 2. Cropping in all study fields, 1987–1994

1993–94	Winter wheat cv Hereward
1992–93	Field beans cv Striker
1991–92	Winter wheat cv Slepjner
1990–91	Linseed cv Antares
1989–90	Winter wheat cv Hereward
1988–89	Spring peas
1987–88	Winter cereal

2.2 Experimental design

Each of the fields (2.3–3.4 ha) was divided into four plots (mean area 0.7 ha), to which four insecticide treatments were randomly assigned (Fig 1). These were cypermethrin 100 g litre^{-1} EC ('Ambush C'®; Zeneca), pirimicarb 500 g kg^{-1} SG ('Aphox'®; Zeneca), chlorpyrifos 480 g litre^{-1} EC ('Spannit'®; PBI) and an unsprayed control. Chlorpyrifos is a chemical to which Collembola are particularly sensitive,[53] and served as a toxic standard. The timing of applications of cypermethin and pirimicarb was agriculturally realistic: in winter wheat, cypermethrin and pirimicarb would be applied as aphicides in June, whilst chlorpyrifos may be used as a diptericide, with potential also for control of aphids.[46]

2.3 Insecticide spray applications

The insecticides were applied in dry weather on 23 June 1994 between 0900 h and 1240 h BST using a Hardi LY 800 tractor-mounted 12-m boom sprayer with a 20-nozzle system of 110° flat fan (F110/1.59/3) nozzles. A volume rate of *c*200 litre ha^{-1} was achieved with an operating pressure of 2.2 bar and forward speed of 6.9 km h^{-1}. To reduce the risk of cross-contamination, insecticide applications were made in ascending order of their known spectrum of toxicity to predatory arthropods, viz pirimicarb (0900–0940 h), cypermethrin (1030–1110 h), chlorpyrifos (1200–1240 h). An interval of *c*50 min was required between insecticides to permit thorough cleansing of the spray tank and transport of the chemical to the field. The time taken to spray an individual plot was *c*8 min.

2.4 Weather conditions

The local screen temperature increased from 20 °C during the application of pirimicarb to 22 °C during chlorpyrifos spraying. Wind speed was mostly 3 to 5 km h^{-1}, initially variable in direction then SE during the application of chlorpyrifos. Dry conditions persisted until heavy rainfall (at times exceeding 32 mm h^{-1}) commenced *c*30 h after the last chlorpyrifos application had been made; the nearest rainfall reading (obtained *c*6 km from the study site) indicated that 49 mm had fallen between 30 and 33 h after the last insecticide applications.

2.5 Arthropod sampling

Ground-dwelling (epigeic) arthropods were sampled using a 'Ryobi' suction sampler similar to one described by Macleod *et al.*[54] On each sampling occasion, five samples, *c*10 m apart, were collected from the centre of each plot, using a central tractor wheeling in the plot as a reference. Each sample (total area 0.052 m^2) comprised five 10-s duration sub-samples (each 104 cm^2) obtained by randomly placing the sampler nozzle in the crop, between wheat plants, within 1.5 m on either side of the tractor wheeling. The pooled sub-samples were transferred *in situ* into vials containing *c*70% methyl alcohol within 2 h of sampling. Samples were collected only when the crop and ground were dry. On each sampling occasion the order in which fields were sampled was varied and sampling of all plots was completed within 3.5 h. Samples were subsequently examined under a binocular light microscope and all arthropods except Acari were removed by hand. Arthropods were identified where possible, with the aid of a compound light microscope if necessary. Sampling was carried out on a total of 10 occasions but insufficient time was available for processing of all samples. Accordingly, only data from 35 days pre- and 10 days post-treatment are reported here.

Collembola were found on maturing ears of wheat during July and August and wheat ears were collected from each plot on 15 August 1994 (immediately before harvest). The purpose was to determine (1) which species were present on the plants, and (2) whether occurrence on wheat ears differed between insecticide treatments. In the centre of each plot, five groups of wheat ears were selected at random. In each group, 20 wheat ears were isolated by bending the surrounding plants away. The isolated plants were then enclosed quickly from above in a large polythene bag and bent at right-angles so as to prevent escape of

Table 3. Agrochemical applications excluding the trial insecticides, 1993–1994

Date	*Type*	*Chemical*
14 Oct 93	Seed dressing	Carboxin + thiobendazole (fungicide, FS)
17 Mar 94	Herbicides	Chlorotoluron (2100 g. AI. ha^{-1}; SC) Mecoprop-p (600 g. AI. ha^{-1}; SL)
28 Apr 94	Growth regulator	Chlormequat + choline chloride + imazaquin (736 + 56 + 1.6 g. AI. ha^{-1}; SL)
	Fungicide	Cyproconazole (40 g. AI. ha^{-1}; SL)
27 May 94	Fungicides	Chlorothalonil + cyproconazole (375 + 40 g. AI. ha^{-1}; SC) Flusilazole (100 g. AI./ha^{-1}; EC) Fenpropidin (187 g. AI. ha^{-1}; EC)

Table 4. ANOVA model for the factors *I* (four levels), *F* (four levels) and *T* (two levels), with five samples *S′* nested within *I* and *F* and cross-factored with *T*

Source of variation	*d f.*	*Mean square*	*F-ratio*
I	3	a	a/h
F	3	b	b/h
I × F	9	c	c/h
T	1	d	d/i
T × I	3	e	e/i
T × F	3	f	f/i
T × I × F	9	g	g/i
within samples + residual:			
S′(I × F)	64	h	
S′(I × F)T	64	i	

arthropods while the ears were excised from the stems using scissors. Groups of wheat ears were sealed in polythene bags and frozen within 2 h of collection. After thawing, wheat ears were dissected in *c*70% methyl alcohol under a binocular light microscope to permit removal by hand of any arthropods present.

2.6 Crop monitoring

Crop growth stage[55] was recorded for 10 randomly-selected plants per field on several occasions during the summer. Densities of wheat stems were estimated using five 50 cm × 50 cm quadrats thrown randomly from the centre of each plot on 7 August. Distributions of weeds and percentage ground cover were recorded in each field on 16 August.

2.7 Statistical analysis

The null hypotheses that pre- to post-treatment changes (−35 days to +10 days) in Collembola abundance were independent of the insecticide treatments, and that effects of insecticide treatments were independent of the field in which they were applied, were tested using a three-way analysis of variance (ANOVA) (Table 4). Insecticide treatment (I), field (F) and time (T) were included as fixed effects because the study did not comprise a random sample of wheat fields. The null hypotheses were tested, respectively, using *F*-ratios for T × I and T × I × F (Table 4). Where treatment effect was significant ($P < 0.05$), 95% confidence intervals for specific T × I contrasts in the ANOVA model were used to infer significant differences between individual insecticides and the unsprayed control. Arthropod counts x were found to exhibit a log-normal distribution and were transformed to $\log_{10}(x+1)$ prior to analysis. Normality of the data after transformation was confirmed graphically using normal quantile plots and homogeneity of variances verified using Cochran's C.[56]

The hypothesis that counts x of arthropods obtained from the ears of wheat on 15 August were independent of insecticide treatment and field was tested by applying a two-way ANOVA to $\log_{10}(x+1)$-transformed counts obtained from five samples (S′) per plot (each sample comprised 20 wheat ears). Insecticide (I) and field (F) were included as fixed effects in the model S′(I × F). The same ANOVA model was used to test the hypothesis that density of wheat stems did not differ between insecticide treatments or fields, using five samples per plot. Density of stems was analysed separately, rather than included as a co-variable in the main analysis of treatment effects because density estimates were made nearly two months after the insecticide treatments were applied. Stem densities were distributed normally and were not transformed prior to analysis.

Partial correlation coefficients (in which effects either of field or treatment were held constant)[56] were calculated to test two hypotheses: (1) that numbers of Collembola on wheat ears at harvest were independent of densities on the ground on 3 July (the latest date for which suction sample data were available); (2) that counts of Collembola were independent of the abundance of Linyphiidae (the most numerous predators of Collembola recorded in the study).

3 RESULTS

3.1 Crop monitoring

Crop development rates were similar in each of the four fields (Fig 2). The density of wheat stems in August was more variable, but not obviously related to the insecticide applications (Fig 3). Weeds were distributed patchily in all fields and within the areas sampled did not exceed 5% ground cover (1.0 m^2 quadrats). The most abundant species were *Avena fatua* L (mainly in Field 3 cypermethrin plot), *Fallopia convulvulus* (L) A Loeve (mainly in Field 4 pirimicarb plot) and *Urtica dioica* L (mainly in Field 2). In all fields, estimated ground cover on 16 August was: wheat 85–90%; bare soil 5–15%; and weeds 0–5%.

3.2 Ground-dwelling Collembola

Assuming that the Collembola captured by suction sampling originated primarily from the ground surface within the area of the sampler nozzle, total collembolan density on the ground in July varied in the unsprayed plots from *c*4900 m^{-2} in Field 1 to *c*19200 m^{-2} in Field 2 (Table 5). Densities of the most numerous individual species differed consider-

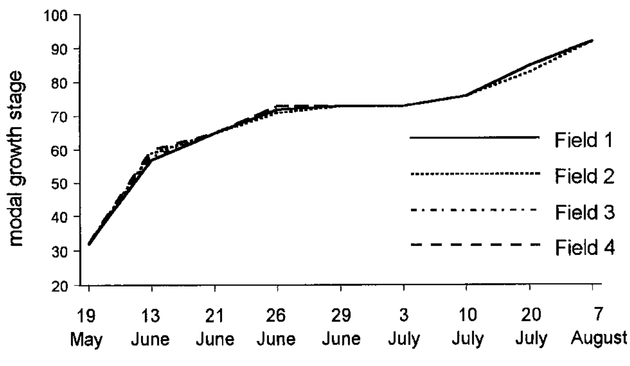

Figure 2. Crop development (decimal growth stage[55]) in the study fields.

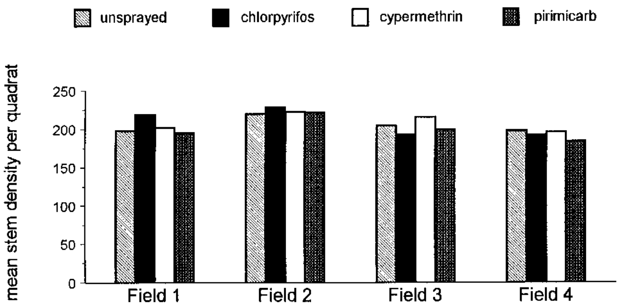

Figure 3. Wheat stem density 0.25 m^{-2} quadrats on 15 August. Differences were not significant for treatment ($F_{3,64}=0.93$, $P \geq 0.05$) or field ($F_{3,64}=2.68$, $P \geq 0.05$).

ably between fields and some taxa (*Deuterosminthurus* spp, *Isotoma viridis, Sminthurinus* spp) were not present in all fields (Table 5).

3.3 Collembola on wheat ears

Ten individual species or genera of Collembola were found on the ears of wheat sampled on 15 August (harvest date), the most frequently occurring species being *Entomobrya multifasciata* (Tullb) (Table 6). Although collembolan densities per plant were low, total Collembola density in the crop canopy may have exceeded 700 m^{-2} (Table 6).

3.4 Effects of insecticides on the ground-dwelling fauna

The null hypothesis that pre- to post-treatment changes in collembolan abundance were independent of insecticide treatment was rejected for eight genera (Table 7). For Collembola, all changes under the chlorpyrifos treatment were negative relative to the unsprayed treatment, whereas under cypermethrin and pirimicarb treatments all changes were positive (Table 7). In six cases significant ($F_{9,64} > 5.00$; $P < 0.001$) interactions between treatment and field ($I \times T \times F$) indicated that the effects of insecticides were not independent of the field in which they were applied (Table 7).

The most numerous predators of Collembola among the arthropods counted in the samples were Linyphiidae and Staphylinidae, with mean counts on 3 July of 6.6–16.6 per suction sample. Linyphiidae were

Table 5. Mean ± SD ($n=5$) suction-sampled Collembola catch (0.052 m^{-2}) in unsprayed plots (3 July)

	Field 1	*Field 2*	*Field 3*	*Field 4*
Isotoma notabilis Schäffer	7.2 (±0.8)	43.6 (±8.4)	4.2 (±3.5)	31.6 (±19.3)
Isotoma viridis Bourlet	0.6 (±0.9)	79.0 (±26.2)	0.8 (±1.1)	0
Isotomurus spp	96.0 (±106.0)	630.4 (±224.0)	715.6 (±248.7)	384.6 (±142.7)
Entomobrya multifasciata (Tullb)	16.2 (±4.5)	10.4 (±5.8)	50.8 (±20.4)	10.8 (±3.1)
Lepidocyrtus spp	64.0 (±40.4)	59.6 (±32.4)	31.8 (±17.6)	3.6 (±2.3)
Pseudosinella decipiens Denis	12.2 (±6.4)	0.2 (±0.4)	2.8 (±2.4)	1.4 (±2.6)
Orchesella villosa (Geoffroy)	0.2 (±0.4)	12.0 (±10.0)	29.2 (±16.4)	0.2 (±0.4)
Heteromurus nitidus (Templeton)	13.2 (±6.7)	11.6 (±10.0)	9.4 (±10.1)	1.6 (±2.6)
Total of sub-order Arthropleona	220.4 (±157.9)	995.6 (±352.4)	850.6 (±265.2)	437.6 (±144.8)
Deuterosminthurus spp	0.4 (±0.5)	0	4.6 (±2.5)	0
Sminthurinus aureus (Lubbock)	0	0.4 (±0.5)	4.4 (±1.7)	10.0 (±3.8)
Sminthurinus elegans (Fitch)	36.2 (±26.7)	0	0.2 (±0.4)	0
Sphaeridia pumilis (Krausbauer)	0.2 (±0.4)	1.2 (±0.8)	18.2 (±14.5)	67.6 (±45.4)
Total of sub-order Symphypleona	37.2 (±26.7)	6.4 (±5.2)	28.2 (±13.9)	78.0 (±48.8)
Total Collembola	257.6 (±183.7)	1002.0 (±354.2)	878.8 (±264.6)	515.6 (±157.8)

Table 6. Occurrence of Collembola on ears of wheat immediately before harvest (15 August)

	Total number (1600 ears)	*Mean number per ear*	*Estimated[a] density m^{-2}*
Entomobrya multifasciata	1067	0.7	534
Isotomurus palustris	225	0.1	113
Lepidocyrtus cyaneus + *L violaceus*	133	0.1	67
Arthropleona nymphs indet	48	<0.1	24
Sminthurinus spp	13	<0.1	6.5
Lepidocyrtus spp indet	4	<0.1	2
Isotoma notabilis	4	<0.1	2
Sphaeridia pumilis	4	<0.1	2
Symphypleona nymphs indet	4	<0.1	2
Heteromurus nitidus	3	<0.1	1.5
Sminthurus viridis	3	<0.1	1.5
Bourletiella hortensis Fitch	1	<0.1	<1
Total Collembola	1509	0.9	755

[a] Based on wheat plant density of 800 m^{-2} (Fig 3).

Table 7. Pre-treatment to post-treatment changes in suction captures of Collembola and predators under three insecticide treatments relative to changes in unsprayed plots

		Direction of change relative to unsprayed control[ab]		
	$F_{3,64}$ ($I \times T$)	*Chlorpyrifos*	*Cypermethrin*	*Pirimicarb*
I notabilis	25.64 ($P<0.001$)	Decrease	Increase	n s
Isotomurus spp.	93.03 ($P<0.001$)	Decrease	n s	n s
E multifasciata	164.0 ($P<0.001$)	Decrease	Increase	n s
Lepidocyrtus spp	54.58 ($P<0.001$)	Decrease[c]	n s	n s
P decipiens	3.55 ($P<0.05$)	n s	n s	n s
O villosa	44.27 ($P<0.001$)	Decrease[c]	n s	n s
H nitidus	4.07 ($P=0.01$)	Decrease	n s	n s
Total Arthropleona	92.65 ($P<0.001$)	Decrease	n s	n s
S aureus	23.04 ($P<0.001$)	Decrease[c]	Increase	n s
S elegans	31.96 ($P<0.001$)	n s	n s	Increase[c]
Sp pumilis	51.37 ($P<0.001$)	Decrease[c]	Increase[c]	n s
Total Sminthurididae	9.47 ($P<0.001$)	Decrease	Increase	Increase
Total Symphypleona	97.63 ($P<0.001$)	Decrease	Increase	n s
Total Collembola	97.15 ($P<0.001$)	Decrease	n s	n s
Linyphiidae	5.22 ($P<0.01$)	Decrease	Decrease	n s
Staphylinidae	12.91 ($P<0.001$)	Decrease	n s	Decrease

[a] Significant changes ($P<0.05$) based on 95% CI for specified contrast with control.
[b] n s Effect not significant ($P \geq 0.05$).
[c] Significant interaction with field but effect consistent in three out of four fields.

negatively affected by chlorpyrifos and cypermethrin whereas Staphylinidae were negatively affected by chlorpyrifos and pirimicarb (Fig 4; Table 7). Correlations between counts of total Linyphiidae and total Collembola in suction samples on 3 July were positive, irrespective of the levels of insecticide treatment included in the correlation analysis (all treatments: $r_{80}=0.24$, $P<0.05$; unsprayed only: $r_{20}=0.47$, $P<0.05$; unsprayed and cypermethrin only: $r_{40}=0.36$, $P<0.05$). No significant correlations between the total linyphiid catch and counts of individual collembolan species were detected.

3.5 Effects of insecticides on crop-inhabiting arthropods

Of 40 arthropod species or groups which were present on the crop at harvest, seven exhibited significant differences in density between insecticide-treated and unsprayed plots (Table 8). These included pest species such as *Sitodiplosis mosellana* (Géhin) (Diptera: orange wheat blossom midge) and Hemiptera (which principally comprised aphids). Significant field-by-treatment interactions occurred for *S mosellana* ($F_{9,64}=2.5$, $P<0.05$) and total Collembola ($F_{9,64}=5.6$, $P<0.001$) but in all fields densities of these species under chlorpyrifos and cypermethrin treatments were consistently lower than those in unsprayed plots. With the exception of Hemiptera, the responses of non-collembolan arthropods to the insecticides were indicative overall of negative effects of chlorpyrifos and cypermethrin. Two groups of Collembola, *Isotomurus* spp and *Entomobrya multifasciata*, exhibited significant effects of the insecticide treatments on their density on wheat ears (Table 8). For *E multifasciata*, the negative influence of chlorpyrifos mirrored an effect of this insecticide on the ground-dwelling cohort (Fig 5). For *Isotomurus* spp, however, insecticide effects on ground-dwelling (Table 7) and crop-climbing individuals (Table 8) were inconsistent.

Two of the arthropod groups which were obtained from wheat ears exhibited significant correlations between densities on the plants on 15 August and densities on the ground (suction sampling) on 3 July. Partial correlation coefficients[56] (ie controlling for effects of field and insecticide) were positive for the collembolan *E multifasciata* ($r_{12}=0.77$, $P=0.001$) and the total Collembola ($r_{12}=0.64$, $P<0.05$).

4 DISCUSSION

4.1 Effects of the insecticides

The results of this work provide evidence for broad-spectrum negative effects of chlorpyrifos on Collembola but not for negative effects of either cypermethrin or pirimicarb. The negative effects of chlorpyrifos are consistent with its known broad spectrum of activity against arthropods.[53] Inclusion of chlorpyrifos in this work revealed differences between fields in collembolan responses (Fig 4), which resulted at least in part from field-to-field variation in abundance (Table 5). It is important to consider variation in the density of vegetation as this may influence arthropod activity and distribution, and also exposure to sprayed pesticides.[46] In the present work the density of crop plants varied between the experimental plots and fields, but the differences were neither consistent in direction nor statistically significant (Fig 3).

Increases in the abundance of Collembola after use of synthetic pyrethroids have been reported in other

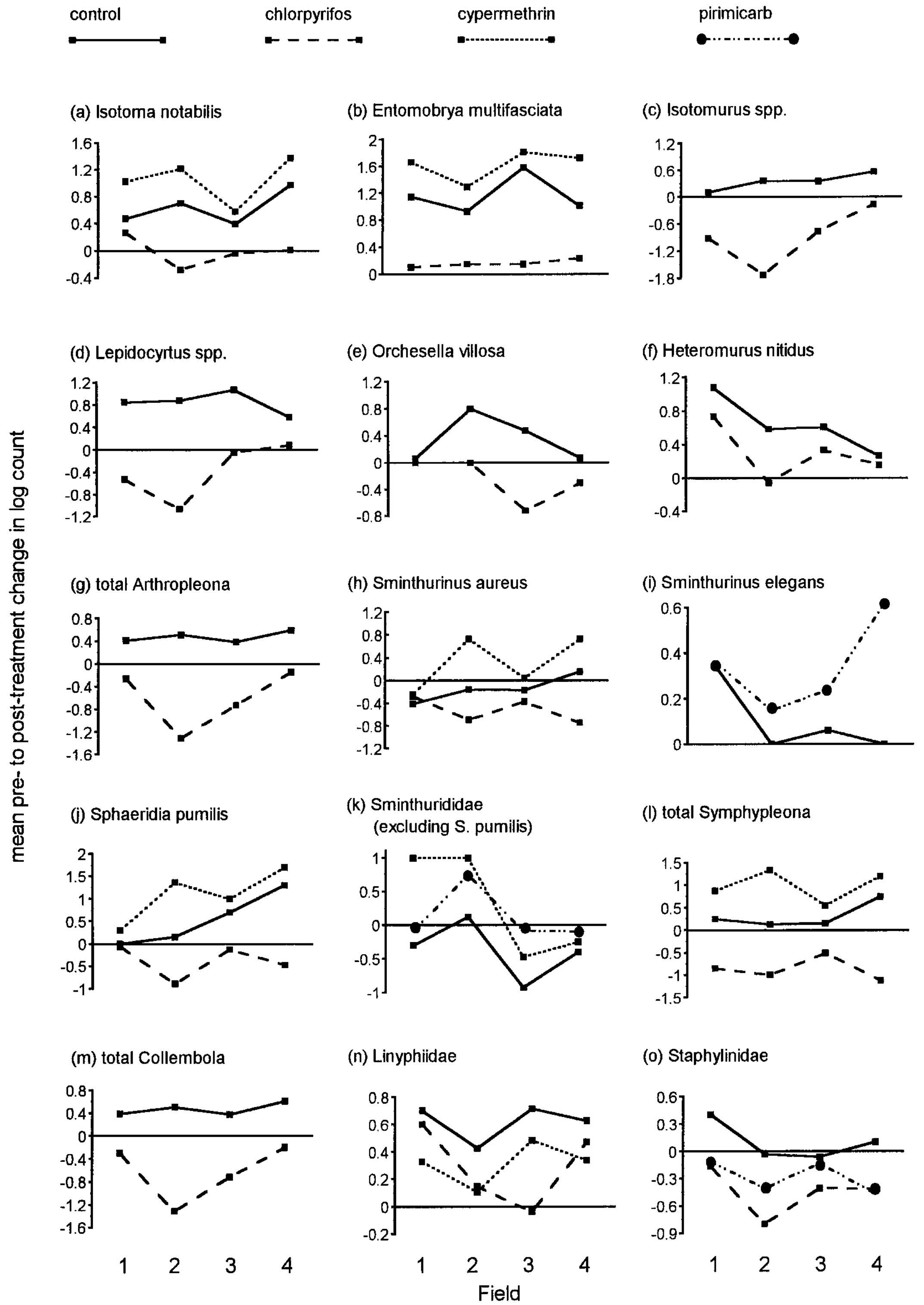

Figure 4. Pre-treatment (−35 days) to post-treatment (+10 days) changes in suction captures of Collembola and predators following insecticide treatment in each of four adjacent fields.

studies.[14,31] A number of possible explanations exist for the relative increases in the abundance of some Collembola species after cypermethrin was applied in the present work. These could include sub-lethal effects on behaviour, or indirect effects resulting from altered interactions with competitors or predators. Negative effects (knockdown or mortality) of synthetic pyrethroid insecticides on potential predators of Collembola are well known,[57] e.g on predacious Acari,[40,58] Araneae[28,32,35,36,38,40,41,59,60] and Carabidae.[17,20,22,28,32,34] Acari were not counted in the present study and Carabidae (mostly *Trechus quadristriatus* Schrank) were rare in suction samples (0.6–4.2 per sample in unsprayed plots), so effects of the cypermethrin application on these arthropods were not investigated. Effects of cypermethrin were negative

Table 8. Differences between insecticide-treated and unsprayed plots in the density of arthropods on wheat ears immediately before harvest

	$F_{3,64}$ (*l*)	Difference relative to unsprayed control[a]		
		Chlorpyrifos	*Cypermethrin*	*Pirimicarb*
Isotomurus spp	3.1 ($P<0.05$)	n s	n s	Higher
Entomobrya multifasciata	81.7 ($P<0.001$)	Lower	n s	n s
All Collembola	30.8 ($P<0.001$)	Lower	n s	n s
Thysanoptera	109.2 ($P<0.001$)	Lower	Lower	n s
Hemiptera	11.4 ($P<0.001$)	n s	Higher	Higher
Sitodiplosis mosellana larvae	38.4 ($P<0.001$)	Lower	Lower	n s
All Diptera larvae	32.6 ($P<0.001$)	Lower	Lower	n s

[a] Tukey HSD test ($a=0.05$); n s denotes $P\geq 0.05$ for the specified contrast.

on Linyphiidae and positive on Collembola, suggesting a possible influence of insecticide use upon linyphiid predation of Collembola; other workers have suggested that use of synthetic pyrethroids may favour increased collembolan abundance through negative effects on predators.[31] In the current work, however, correlations between the abundance of Collembola and Linyphiidae were positive, indicating that high collembolan abundance did not consistently occur in samples with low linyphiid density. Further work would be needed to test properly the hypothesis that positive effects of cypermethrin upon Collembola result from an indirect effect on predation.

A positive effect of pirimicarb upon abundance of Collembola was only detected in two groups, viz *Sminthurinus elegans* and the family Sminthurididae (which excludes *S elegans*) (Fig 4; Table 7). A lack of negative effects of pirimicarb on Collembola is consistent with its selectivity as an aphicide,[57] ie its relatively narrow spectrum of effects on non-target arthropods.[17–19,23,25,39,61]

4.2 Crop-inhabiting arthropods

Species of Collembola which occur on wheat plants (Table 6) could be at risk of direct exposure to pesticide sprays and to residues present on the crop.[46] In this study, the presence of arthropods on the wheat plants was quantified only at harvest and so the times at which different species colonised the foliage are not known. A positive correlation between the density of *E multifasciata* on the crop in August and captures from the ground (suction samples) in July indicates that spatial distributions of crop-dwelling and ground-inhabiting cohorts of the population were not independent of one another. Effects of chlorpyrifos in the crop canopy were similar to those observed on ground-dwelling insects and clearly apparent nearly eight weeks after the insecticide application (Fig 5). Slow recovery of *E multifasciata* after use of chlorpyrifos[62] would be expected if the species has an inherently low dispersal ability. However, eight weeks is a relatively short time in which to expect arthropod recovery from effects of a chlorpyrifos spray,[63,64] lack of recovery within 0.8–1.1 years would not be unexpected given the toxicity and persistence of the chemical.[53] The negative effects of chlorpyrifos and cypermethrin on canopy-inhabiting arthropods persisted even though heavy rainfall had occurred within 32 h of the insecticide applications. Adverse effects on predatory arthropods, particularly of synthetic pyrethroid insecticides, are liable to be reduced substantially if rain falls soon after an application.[35,59]

4.3 Comparison with in-situ bioassays

During the experiment, an additional method for assessing effects of the insecticides on Collembola, using in-situ field bioassays, was tested in one of the four study fields.[46] The technique involved the collection of insecticide residues from Field 2 and

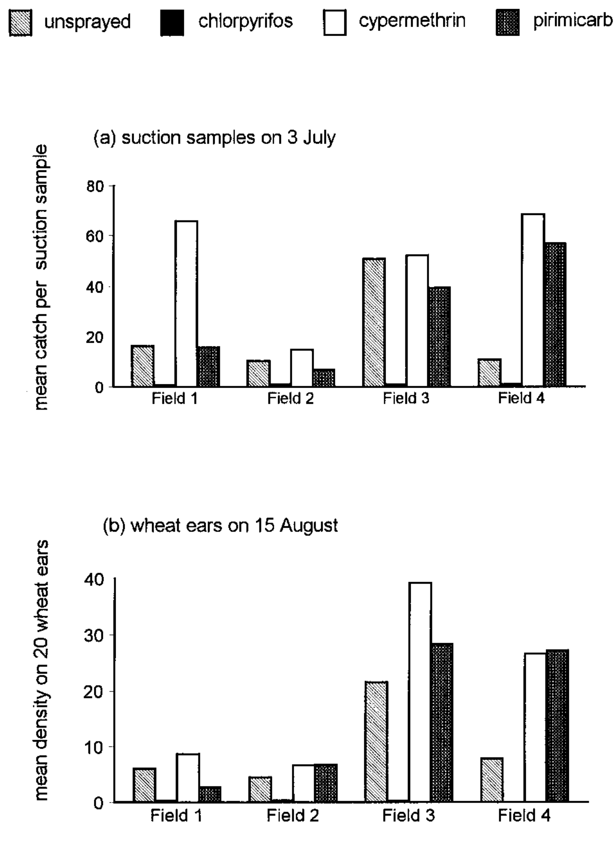

Figure 5. Captures of *Entomobrya multifasciata* obtained from suction samples and wheat ears under three insecticide treatments applied on 23 June.

subsequent exposure of Collembola to the residues under standard laboratory conditions. The bioassay approach permitted species which were not present, or rare, in suction samples to be exposed to realistic insecticide residues. It also enabled effects of manipulating exposure and soil type to be investigated. Results of the bioassay work revealed toxicity of chlorpyrifos, but not of cypermethrin or pirimicarb, to *Folsomia candida* Willem, *Isotoma viridis* Bourlet *Isotomurus palustris* (Muller) and *Sminthurus viridis* L and thus complement the results obtained from sampling arthropods in the field. However, a limitation of the field bioassay approach was that it could not detect the increases in abundance of some Collembola species caused by cypermethrin or pirimicarb. Whether the bioassay method could be improved to include such positive effects of insecticides would depend on the causal mechanism of the effects, which at present is unknown. Despite these limitations, there are several advantages to using a bioassay approach[46] and the conclusions obtained using in-situ bioassays, ie that cypermethrin and pirimicarb were not harmful to four species of Collembola, remain valid.

4.4 Design of the experiment

Much emphasis has been placed on efforts to integrate realistic spatial scales of study with the need for statistical replication.[23,36,65–67] As the area available for field experiments is usually limited, e g by the size of fields, replication places a constraint on the size of experimental plots that can be accommodated. This is problematic because, among predatory species, the rate of recolonisation (and hence recovery) is related inversely to the plot size.[68–70] Effects of pesticides on Collembola have been detected using particularly small plots ($10\,m \times 10\,m$,[40] $2\,m \times 3\,m$,[71] or $1\,m \times 1\,m$[72]), suggesting that at least some (especially euedaphic) collembolan species may have relatively low dispersal ability. However, it has been established that the relationship between plot size and recovery rate of predators also affects predation pressure at different distances into a pesticide-treated area.[70,73] For ecological realism therefore, the spatial scale of study should be appropriate not only for Collembola but also for their predators, some of which have relatively high rates of dispersal.[74]

Assuming that estimates of arthropod recovery rates after use of broad-spectrum insecticides for carabid beetles[68] and linyphiid spiders[69] are broadly applicable to the current work, recolonisation of predators over a distance of 24 m (the shortest distance between a sampling location and potential source of recolonists in the current work) would not have been expected within +10 days of treatment.

4.5 Arthropod spatial variability

The inclusion of several fields in the work was an advantage because it yielded information on field-to-field variation in faunal abundance and insecticide effects. Spatial heterogeneity of faunas is a particular problem in ecotoxicological studies, where results obtained from individual fields may be difficult to interpret if vulnerable species are absent from some fields (Table 6). Two collembolan taxa which were particularly heterogeneous in their distribution between fields, *Isotoma viridis* and *Deuterosminthurus* spp (Table 5), were entirely absent from samples collected from chlorpyrifos-treated plots on 3 July but too rare in three out of four fields to warrant inclusion in the analysis.

4.6 Implications for the use of indicator species

None of the Collembola species studied was negatively affected by cypermethrin. In terms of the detection of negative effects of synthetic pyrethroid insecticides, other arthropods such as Linyphiidae[35,75] would be more appropriate as bioindicators. Collembola are, however, clearly susceptible to organophosphorus insecticide use,[44,62] possibly to a greater extent than predatory arthropods.[53] A combination of Collembola and Linyphiidae could have potential value for the detection of negative insecticide effects in agricultural systems where both synthetic pyrethroids and organophosphates are used widely, as occurs in UK arable farming.[43]

The spatial variability of Collembola is an unwanted attribute when it comes to the selection of bioindicator species, but heterogeneous spatial and temporal distributions in arable land are also found among predatory arthropod species,[76] including recommended bioindicator species such as the carabid beetle *Pterostichus* (=*Poecilus*) *cupreus* L.[77,78] The use of 'guilds' of species with similar ecological characteristics[65] is one possible approach for dealing with the problem of spatial variability; the more species that such a 'guild' contains, the greater likelihood that the guild will be represented in a study field. Given the relative paucity of information on collembolan ecology in arable habitats it is difficult to assign species of Collembola to guilds *a priori* on the basis of ecological characteristics. However, species could instead be grouped according to their responses to pesticides, in which case a suitable guild for the detection of either negative effects of chlorpyrifos *or* positive effects of cypermethrin could include *I notabilis* and *E multifasciata* (Table 7). Ideally, proposals for the use of particular species as bioindicators should draw upon information from as many pesticide studies as possible. A previous attempt at identifying species of potential value as bioindicators of negative effects of pesticide use[44] yielded a number of species which were not encountered during the present work, underlining the need for a multi-species approach with Collembola for detecting non-target pesticide effects.

5 CONCLUSIONS

Summer aphicide sprays of cypermethrin and pirimicarb in winter wheat were not harmful to any species of epigeic Collembola. Cypermethrin increased abundance of several species relative to densities in unsprayed plots whereas most of the species captured were unaffected by pirimicarb. Results from this and other studies suggest that a stimulatory effect of synthetic pyrethroids on Collembola might be a general phenomenon but the causal mechanism is not at present known. The possibility that increased collembolan abundance was caused by negative effects of synthetic pyrethroids on predatory arthropods warrants further investigation because many predators of Collembola are also natural enemies of crop pests. Effects of chlorpyrifos were consistently negative and persisted at least until harvest. Despite apparent homogeneity of the site, some species apparently susceptible to chlorpyrifos were not present in all fields, endorsing the need for a multi-species approach to the use of Collembola as bioindicators of pesticide effects in field studies.

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