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Collembola and macroarthropod community responses to carbamate, organophosphate and synthetic pyrethroid insecticides: Direct and indirect effects

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Direct and indirect insecticide effects differ among closely-related arthropod taxa; resurgence of Collembola may occur widely after synthetic pyrethroid insecticide applications.

Abstract

Non-target effects on terrestrial arthropod communities of the broad-spectrum insecticides chlorpyrifos and cypermethrin and the selective insecticide pirimicarb were investigated in winter wheat fields in summer. Effects of chlorpyrifos on arthropod abundance and taxonomic richness were consistently negative whereas effects of cypermethrin were negative for predatory arthropods but positive for soil surface Collembola. Pirimicarb effects were marginal, primarily on aphids and their antagonists, with no effect on the Collembola community. Collembola-predator ratios were significantly higher following cypermethrin treatment, suggesting that cypermethrin-induced increases in collembolan abundance represent a classical resurgence. Observations in other studies suggest Collembola resurgences may be typical after synthetic pyrethroid applications. Collembola responses to insecticides differed among species, both in terms of effect magnitude and persistence, suggesting that coarse taxonomic monitoring would not adequately detect pesticide risks. These findings have implications for pesticide risk assessments and for the selection of indicator species.

Keywords: Indicators; Non-target effects; Principal response curves; Resurgence; Risk assessment

1. Introduction

Synthetic pyrethroids are the most widely used class of insecticide in European agriculture (in 2004, they accounted for 75% (4.9 million ha) of the insecticide-treated arable crop area in Britain, with cypermethrin being the most widely-used individual insecticide (2.0 million ha); UK Government, Pesticide Usage Survey Statistics). A selective carbamate aphicide, pirimicarb, is also used extensively in Europe (in 2004 it accounted for ca 7% (0.4 million ha) of the area of insecticide-treated arable crops in Britain). Non-target effects of cypermethrin and pirimicarb on terrestrial arthropod populations have been studied in detail for predatory macroarthropods (e.g. Coleoptera and Araneae) but relatively little is known about their effects on Collembola (springtails). Epigeic (soil surface) Collembola are abundant in arable fields (where densities may exceed 10,000 m^{-2}) and are likely to be exposed to sprayed and soil-applied pesticides. They are also important in food webs, as fungivores and as prey for a wide range of specialist and generalist predators, including Arachnida and Coleoptera (Hopkin, 1997).

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The majority of literature suggests that Collembola abundance is generally not affected adversely by synthetic pyrethroid insecticides in arable agriculture or forestry field studies (Heungens and van Daele, 1979; Hill, 1985; Shires, 1985; Inglesfield, 1989; Smart et al., 1989; Dewar et al., 1990; Huusela-Veistola et al., 1994; Bishop et al., 1998; Frampton, 1999; Baker et al., 2002). Synthetic pyrethroid applications decreased Collembola catches in barley (Huusela-Veistola et al., 1994) and beans (Tripathi and Sharma, 2005) whereas in hops, spatially inconsistent effects occurred (Filser and Nagel, 1993). However, not all studies were clearly reported and in some cases it is unclear whether the pesticide treatments were appropriately replicated. In four studies, Collembola catches increased after applications of synthetic pyrethroids in forest plots (Funke et al., 1994; Holopainen and Rikala, 1995) and arable crops (Gimeno and Perdiguer, 1995; Frampton, 1999). With the exception of three studies (Funke et al., 1994; Filser and Nagel, 1993; Frampton, 1999), responses of individual Collembola species were not investigated in detail; usually only the total Collembola catch was reported.

Hardly any studies of the effects of pirimicarb on natural populations of Collembola have been published, with only one detailed field investigation (Frampton, 1999) and a study in which individual species were introduced into microcosms (Løkke, 1985). In the field study, pirimicarb had no significant effect on the abundance of 12 taxa but increased catches of two taxa. However, the effects of pirimicarb were based on the change in abundance from one pre-treatment sampling to one post-treatment sampling and appear sensitive to the statistical method of analysis (see Section 4). In the microcosm study, pirimicarb had a negative effect on the abundance of one introduced species (*Folsomia fimetaria*) but only on one sampling date (70 days after treatment), with statistical significance of the effect dependent on the presence of data outliers (Løkke, 1995).

This paper provides a more detailed analysis of the field study carried out in 1994 by Frampton (1999), which extends the data available on effects of cypermethrin and pirimicarb from two to seven sampling dates. Analysis of archived arthropod samples from the original study has been made possible by the provision of additional resources (see Acknowledgements). Since the original field study was carried out there has been relatively little new information in the literature about effects of cypermethrin and pirimicarb on Collembola, although some contract testing laboratories have observed increased collembolan abundance after synthetic pyrethroid applications (Ecotox Ltd., Tavistock, UK; ECT Ökotoxikologie GmbH, Flörsheim, Germany; Mambo-Tox, Southampton, UK; personal communications). Such increased abundance reflects indirect effects that might be more widespread than the published literature suggests. Understanding the extent of such indirect effects is important in risk assessment because they cannot be predicted using single-species toxicity tests (Wiles and Frampton, 1996).

Collembola and macroarthropod community responses to cypermethrin and pirimicarb are presented in detail, to clarify the persistence and taxonomic spectrum of effects observed previously (Frampton, 1999). Effects of the broad-spectrum organophosphorus insecticide chlorpyrifos are also included for comparison. To our knowledge, this work represents the most detailed examination of how natural Collembola and terrestrial macroarthropod communities respond to cypermethrin and pirimicarb. The implications of the findings are discussed in terms of whether individual Collembola species or groups might be useful as indicators of adverse pesticide effects on their predators.

2. Methods and materials

Full details of the study site, experiment design, insecticide applications and arthropod sampling are given by Frampton (1999). The study was carried out in south-east England (51° 16′ N, 0° 23′ E) during summer 1994 and employed four insecticide treatments (unsprayed, chlorpyrifos, cypermethrin and pirimicarb). Chlorpyrifos was included as a toxic reference treatment. Each pesticide was applied to plots (0.58 to 0.85 ha) in four contiguous fields of winter wheat such that each field contained one randomized replicate of each treatment. These four fields had previously been treated together as a single management unit and were selected in view of their apparent homogeneity of soils, previous cropping and husbandry.

2.1. Insecticide applications

Insecticides were applied on 23 June using a tractor-mounted boom sprayer, according to label recommendations for winter wheat (chlorpyrifos (‘Spannit’®; PBI): 480 g a.i. ha^{-1}; cypermethrin (‘Ambush C’®; Zeneca): 25 g a.i. ha^{-1}; pirimicarb (‘Aphox’ ®; Zeneca): 40 g a.i. ha^{-1}). These resulted in homogeneous spray deposition rates on the soil surface (0.11 to 0.13 µl cm^{-2}) that did not differ between treatments (Wiles and Frampton, 1996). The wind speed during applications was mostly 3 to 5 km h^{-1} and screen temperature 20–22 °C. A notable feature of the weather is that approximately 30 h after the insecticide applications a ca 3-h period of heavy rainfall (total ca 40 mm) occurred.

2.2. Arthropod sampling

Arthropods were sampled during dry weather using a Ryobi suction sampler (Macleod et al., 1994) on seven sampling occasions (35 d and 2 d pre-treatment and 6 d, 10 d, 17 d, 27 d and 44 d after treatment; the last sampling was one week before crop harvest). On each occasion, five samples (0.052 m^2) were taken randomly from the centre of each plot (each sample was obtained by pooling five randomly-placed 104-cm^2 sub-samples; Frampton, 1999). Samples were preserved in methylated spirit and stored in darkness below 15 °C prior to sorting. Due to the large number of specimens collected, identification of all individual species was not feasible. Thus, *Isotoma viridis* and *I. anglicana* (Isotomidae) are reported together as ‘*Isotoma viridis* group’, whilst *Sminthurinus aureus* and *Sminthurinus niger* (Sminthuridae) are reported together as ‘*Sminthurinus aureus* group’. The name *Isotoma notabilis* used previously (Frampton, 1999) is now considered a junior synonym of *Parisotoma notabilis* (Hopkin, in press). Macroarthropods in the samples were also identified to enable effects of the insecticides on the collembolan and macroarthropod communities to be compared. A notable exception is that it was not feasible to record predatory Acari due to their low abundance in the majority of samples.

2.3. Data analysis

All statistical analyses were carried out on normalised data, using the log $(x + 1)$ of arthropod counts x. Community responses to the insecticide treatments were analysed using the software program CANOCO 4 (Ter Braak and Šmilauer, 1998) to generate Principal Response Curves (PRC) for Collembola and macroarthropods. For each species (k), date (d) and treatment (t), the response (T_{dtk}) was modelled as a multiple (the species weight, b_k) of one basic community response pattern (c_{dt}), with the unsprayed treatment nominated as

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a reference ($c_{dt} = 0$ for all t). Differences in c_{dt} between treatments and dates (plotted as a PRC diagram) indicate the changes in fitted relative abundance for the overall community (van den Brink and Ter Braak, 1999). A null hypothesis that the PRC diagram does not display the treatment variance ($T_{dtk} = 0$ for all t, d, k) was tested using an F-type statistic (Ter Braak and Šmilauer, 1998). Permutation tests within sampling dates were also used to test the null hypothesis that the principal response c_{dt} does not differ between treatments (van den Brink and Ter Braak, 1999).

Species weights obtained in PRC analysis could, in theory, be used to select taxa to analyse in more detail (e.g., using univariate statistics). However, taxa that are sensitive to the insecticides might have relatively low species weights in some situations (for example, if the response pattern of sensitive species follows a different temporal pattern to the fitted community response). Univariate analyses were therefore also carried out, separately for each taxon that had a mean count per sample ≥1 ($n = 20$). For each sampling date, null hypotheses that counts of arthropods were independent of insecticide treatment and effects of insecticide treatment were independent of field were tested using an analysis of variance model with treatment (fixed, $n = 2$: unsprayed v. insecticide), field (random, $n = 4$) and treatment × field. For cypermethrin, a null hypothesis that the log ratio of Collembola to predators was independent of insecticide treatment was tested using the same model.

3. Results

Altogether, 267,006 arthropods were identified, representing 23 Collembola taxa and 34 macroarthropod taxa (Appendix 1). Collembola make up 91% of the catch, with the most abundant collembolan taxon, *Isotomurus* spp., contributing 48% of all data (on average 18,252 per m^2). The most abundant of the macroarthropods were Linyphiidae (2.4% of all data, on average 932 per m^2). Summary statistics are given (in a format suitable for quantitative meta-analysis) both for statistically significant treatment effects (Tables 1–3) and for non-significant effects (Supplementary Tables S1–S3). Differences between the unsprayed and insecticide treatments were not statistically significant on either of the pre-treatment sampling dates.

3.1. Overall response of the collembolan community

The PRC analysis (Fig. 1a) was applied to all species, including those too rare to analyse individually with univariate statistics (Appendix 1). Of the total variance in the data set, 41% is explained by time, 30% by field and 29% by treatment. The PRC diagram displays the first PRC axis, which explains a significant part of the treatment variance (74%; $P = 0.03$). The second PRC axis (omitted) did not display a significant part of the treatment variance (11%; $P > 0.05$). The PRC diagram clearly shows a pronounced negative effect of chlorpyrifos and lack of any effect of pirimicarb on the overall collembolan community. An overall positive effect of cypermethrin on Collembola is also clearly displayed by the PRC diagram, but is not statistically significant at the community level (probably because the most abundant group, *Isotomurus* spp., was not significantly affected by cypermethrin). Taxa with the highest positive weights (b_k) most closely follow the overall fitted community response indicated in the PRC diagram (*S. pumilis*, *E. multifasciata*, *Lepidocyrtus cyaneus* 'group'), whereas negative species weights indicate a response opposite to that displayed in the PRC diagram (e.g. Corticariinae in Fig. 1b). Interpretation of species weights is explained further by van den Brink and Ter Braak (1999).

3.2. Responses of individual collembolan taxa

The null hypothesis that collembolan catches were independent of the chlorpyrifos treatment was rejected for 11 taxa and also for the collembolan taxonomic richness (Table 1).

With the exception of *Pseudosinella alba*, Collembola were consistently least abundant in chlorpyrifos-treated plots, although the differences were not statistically significant for *Sminthurinus aureus* group, which occurred at relatively low abundance in unsprayed plots (Fig. 2). The substantial reduction of collembolan abundance (Fig. 1m) and taxonomic richness (Fig. 2n) following chlorpyrifos application without recovery up to 44 days after treatment is typical of the effects of this insecticide on epigeic Collembola (Frampton, 2002).

For cypermethrin, the null hypothesis of no treatment effects was rejected for six collembolan taxa (Table 2). Three taxonomically unrelated Collembola species exhibited significant responses to cypermethrin, in all cases with abundance increasing relative to unsprayed plots. The Arthropleona (elongate) species *Entomobrya multifasciata* showed an apparently increasing effect of cypermethrin with time, which was statistically significant for the last 3–4 weeks of the study, with abundance approximately twice that in the unsprayed treatment (Fig. 2a). Unhatched eggs of *Entomobrya multifasciata* were found more often in samples from cypermethrin-treated plots than those of the other treatments, but numbers were too low for statistical analysis. In contrast, the Symphypleona (round springtails) *Sminthurinus aureus* group and *Sphaeridia pumilis* showed a clear and statistically significant positive numerical response to cypermethrin soon after treatment (Fig. 2j,k) that persisted for approximately two weeks in *S. pumilis* and the total Symphypleona catch (Fig. 2l). Although the effect was transient, cypermethrin caused a ca five-fold increase in abundance of the Symphypleona. Cypermethrin effects were also statistically significant for the total collembolan catch (Fig. 2m) but not taxonomic richness (Fig. 2n).

No collembolan taxa exhibited statistically significant effects of pirimicarb (Table 3). For *E. multifasciata* and *S. pumilis* in particular (Fig. 2a,k), catches were remarkably similar in the pirimicarb-treated and unsprayed plots. Overall, pirimicarb had no effect on the total collembolan catch (Fig. 2m) or taxonomic richness (Fig. 2n).

3.3. Overall response of the macroarthropod community

PRC analysis for macroarthropods (Fig. 1b) included all macroarthropod taxa (Appendix 1) except aphids. For clarity in the diagram, aphids are excluded from the analysis presented in Fig. 1b, as their inclusion would result in almost identical values of c_{dt} for cypermethrin and pirimicarb on all post-treatment sampling dates. Of the total variance, 60% is explained by time, 25% by field and 15% by treatment. The PRC diagram displays a significant part of the treatment

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Table 1
Summary of statistically significant effects of chlorpyrifos, represented by the mean of $\log_{10}(x+1)$-transformed catches (x) in unsprayed (Un) and chlorpyrifos-treated (Ch) areas and the SE of the mean difference (SED) ($n = 20$; 4 replicate fields × 5 samples per field)

Taxon	Time after insecticide treatment														
	6 d			10 d			17 d			27 d			44 d		
	Un	Ch	(SED)	Un	Ch	(SED)	Un	Ch	(SED)	Un	Ch	(SED)	Un	Ch	(SED)
Collembola															
Entomobrya multifasciata	1.31	0.24	(0.10)**	1.24	0.26	(0.15)**	1.44	0.27	(0.10)**	1.80	0.31	(0.15)**	1.91	0.37	(0.16)**
Lepidocyrtus cyaneus group	1.65	0.61	(0.19)*	1.38	0.46	(0.28)*	1.62	0.42	(0.26)*	1.79	0.23	(0.30)*	1.90	0.33	(0.29)*
Heteromurus nitidus	0.97	0.70	(0.20)	0.79	0.48	(0.26)	0.89	0.42	(0.23)	1.04	0.47	(0.22) (*)	0.99	0.50	(0.21)
Total Entomobryidae	1.95	1.15	(0.14)*	1.86	1.10	(0.24)*	2.04	0.93	(0.20)*	2.27	0.84	(0.29)*	2.35	0.95	(0.31)*
Isotomurus spp.	2.43	1.29	(0.14)**	2.50	1.32	(0.24)*	2.56	1.05	(0.22)**	1.83	0.86	(0.34)*	1.33	0.31	(0.25)*
Parisotoma notabilis	1.47	0.57	(0.20)*	1.14	0.44	(0.28)	1.23	0.53	(0.35)	1.45	0.64	(0.32)	1.59	0.83	(0.25)
Total Isotomidae	2.49	1.35	(0.14)**	2.56	1.47	(0.25)*	2.61	1.24	(0.23)**	2.06	0.88	(0.28)*	1.91	0.95	(0.19)*
Total Arthropleona	2.64	1.60	(0.15)**	2.70	1.70	(0.25)*	2.75	1.43	(0.23)*	2.52	1.20	(0.21)**	2.50	1.28	(0.23)*
Sphaeridia pumilis	0.73	0.06	(0.16)*	0.82	0.05	(0.37)	0.89	0.06	(0.39)	0.89	0	(0.35)	0.86	0.02	(0.33)
Total Symphypleona	1.26	0.17	(0.06)***	1.38	0.18	(0.24)*	1.35	0.08	(0.25)*	1.47	0.14	(0.22)**	1.57	0.32	(0.15)**
Total Collembola	2.66	1.61	(0.15)**	2.74	1.70	(0.24)*	2.78	1.44	(0.21)**	2.57	1.22	(0.21)**	2.58	1.31	(0.22)**
Collembola taxon richness	1.04	0.82	(0.05)*	1.13	0.86	(0.06)*	1.07	0.77	(0.07)*	1.12	0.76	(0.08)*	1.13	0.85	(0.08)*
Macroarthropods															
Aleocharinae	0.53	0.15	(0.05)**	0.55	0.28	(0.07)*	0.48	0.25	(0.13)	0.30	0.23	(0.10)	0.21	0.17	(0.09)
Total adult Staphylinidae	0.58	0.17	(0.07)*	0.66	0.36	(0.07)*	0.63	0.36	(0.12)	0.56	0.47	(0.05)	0.54	0.50	(0.06)
Tachyporus spp. larvae	0.67	0.29	(0.12)	0.68	0.54	(0.05)	0.52	0.35	(0.04)*	0.32	0.28	(0.05)	0.12	0.16	(0.11)
Lathridius spp.	0	0		0.08	0	(0.06)	0.08	0	(0.04)	0.14	0.02	(0.03)*	0.17	0.03	(0.05)
Total adult Coleoptera	0.75	0.32	(0.14)	0.87	0.61	(0.14)	0.82	0.56	(0.08)	0.83	0.71	(0.03)*	0.88	0.83	(0.08)
Cecidomyiidae	1.04	0.45	(0.18)*	1.41	0.70	(0.14)*	1.28	0.69	(0.14)*	0.58	0.50	(0.07)	0.40	0.32	(0.04)
Diptera excl. Cecidomyiidae	0.52	0.26	(0.06)*	0.83	0.64	(0.13)	0.68	0.48	(0.11)	0.41	0.43	(0.05)	0.34	0.37	(0.12)
Total Diptera	1.16	0.58	(0.14)*	1.51	0.98	(0.05)**	1.37	0.89	(0.04)**	0.76	0.72	(0.07)	0.60	0.54	(0.07)
Aphididae	0.98	0.56	(0.07)**	1.03	0.79	(0.13)	0.98	0.81	(0.17)	0.24	0.18	(0.04)	0.06	0.05	(0.07)
Delphacidae	0.59	0.16	(0.13)*	0.83	0.21	(0.01)***	0.98	0.25	(0.04)***	0.60	0.13	(0.07)**	0.52	0.08	(0.10)*
Total Homoptera	1.13	0.62	(0.06)**	1.24	0.86	(0.09)*	1.29	0.90	(0.07)**	0.68	0.29	(0.08)*	0.55	0.13	(0.14)
Thysanoptera	0.65	0.70	(0.09)	0.93	0.75	(0.02)**	0.78	0.60	(0.02)**	0.83	0.62	(0.14)	0.88	0.59	(0.18)
Hymenoptera Parasitica	0.54	0.31	(0.07)*	0.86	0.43	(0.05)**	0.92	0.56	(0.03)***	0.79	0.39	(0.06)**	0.75	0.30	(0.06)**
Araneae Linyphiidae	0.79	0.64	(0.11)	0.97	0.80	(0.08)	1.06	0.86	(0.09)	1.48	1.27	(0.06)*	1.43	1.25	(0.08)
Total macroarthropod catch	1.67	1.26	(0.06)**	1.92	1.52	(0.05)**	1.89	1.50	(0.04)**	1.80	1.56	(0.02)**	1.78	1.53	(0.04)**
Collembola + macroarthropods															
Total catch	2.72	1.77	(0.11)**	2.81	1.93	(0.18)*	2.85	1.78	(0.18)**	2.65	1.73	(0.12)**	2.64	1.75	(0.12)**
Overall taxonomic richness	1.31	1.12	(0.02)**	1.39	1.21	(0.03)*	1.35	1.16	(0.04)*	1.40	1.18	(0.03)**	1.40	1.20	(0.55)

Asterisks show effects identified in analysis of variance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). (*) indicates an interaction between treatment and field but where the treatment effect was consistent and significant ($P < 0.05$) in three out of four fields. Summary data for taxa that did not exhibit significant effects of chlorpyrifos are given in Supplementary Table S1.

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Table 2

Summary of statistically significant effects of cypermethrin, represented by the mean of $\log_{10}(x+1)$-transformed catches (x) in unsprayed (Un) and cypermethrin-treated (Cy) areas and the SE of the mean difference (SED) ($n = 20$; 4 replicate fields × 5 samples per field)

Taxon	Time after insecticide treatment														
	6 d			10 d			17 d			27 d			44 d		
	Un	Cy	(SED)	Un	Cy	(SED)	Un	Cy	(SED)	Un	Cy	(SED)	Un	Cy	(SED)
Collembola															
Entomobrya multifasciata	1.31	1.54	(0.19)	1.24	1.63	(0.17)	1.44	1.88	(0.14)	1.80	2.14	(0.10)*	1.91	2.24	(0.06)*
Total Entomobryidae	1.95	2.09	(0.14)	1.86	1.22	(0.14)	2.04	2.25	(0.14)	2.27	2.49	(0.07)	2.35	2.58	(0.07)*
Sminthurinus aureus 'group'	0.52	1.13	(0.14)*	0.46	0.97	(0.24)	0.52	0.98	(0.33)	0.63	0.78	(0.19)	0.67	0.89	(0.23)
Sphaeridia pumilis	0.73	1.35	(0.18)*	0.82	1.54	(0.33) (*)	0.89	1.54	(0.31) (*)	0.89	1.25	(0.20)	0.86	1.13	(0.24)
Total Symphypleona	1.26	1.77	(0.17)	1.38	2.04	(0.26) (*)	1.35	2.04	(0.29) (*)	1.47	1.74	(0.14)	1.57	1.67	(0.11)
Total Collembola	2.66	2.82	(0.12)	2.74	2.89	(0.13)	2.78	2.90	(0.10)	2.57	2.75	(0.05)*	2.58	2.76	(0.06)*
Macroarthropods															
Cecidomyiidae	1.04	0.29	(0.14)*	1.41	0.51	(0.07)**	1.28	0.50	(0.05)***	0.58	0.43	(0.13)	0.40	0.22	(0.13)
Diptera excl. Cecidomyiidae	0.52	0.23	(0.09)*	0.83	0.55	(0.13)	0.67	0.53	(0.09)	0.41	0.38	(0.08)	0.34	0.33	(0.18)
Total Diptera	1.16	0.43	(0.13)*	1.51	0.78	(0.08)**	1.37	0.77	(0.02)***	0.76	0.62	(0.12)	0.60	0.44	(0.19)
Delphacidae	0.59	0.31	(0.14)	0.83	0.63	(0.07)	0.98	0.78	(0.06)*	0.60	0.45	(0.06)	0.52	0.36	(0.06)
Thysanoptera	0.65	1.21	(0.09)**	0.93	0.92	(0.06)	0.78	0.79	(0.07)	0.83	0.48	(0.07)*	0.88	0.42	(0.11)*
Hymenoptera Parasitica	0.54	0.52	(0.09)	0.86	0.70	(0.04)*	0.92	0.75	(0.05)*	0.79	0.60	(0.13)	0.75	0.58	(0.12)
Araneae Linyphiidae	0.79	0.42	(0.19)	0.97	0.86	(0.65)	1.06	0.79	(0.06)*	1.48	1.35	(0.06)	1.43	1.36	(0.06)
Total macroarthropod catch	1.67	1.59	(0.04)	1.92	1.78	(0.03)*	1.89	1.75	(0.02)**	1.80	1.65	(0.02)**	1.78	1.62	(0.04)
Collembola + macroarthropods															
Total catch	2.72	2.85	(0.11)	2.81	2.93	(0.10)	2.85	2.94	(0.08)	2.65	2.78	(0.04)	2.64	2.79	(0.06)

Asterisks show effects identified in analysis of variance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). (*) indicates an interaction between treatment and field but where the treatment effect was consistent and significant ($P < 0.05$) in three out of four fields. Summary data for taxa that did not exhibit significant effects of cypermethrin are given in Supplementary Table S2.

Table 3
Summary of statistically significant effects of pirimicarb, represented by the mean of $\log_{10}(x + 1)$-transformed catches (x) in unsprayed (Un) and pirimicarb-treated (Pi) areas and the SE of the mean difference (SED) ($n = 20$; 4 replicate fields × 5 samples per field)

Taxon	Time after insecticide treatment														
	6 d			10 d			17 d			27 d			44 d		
	Un	Pi	(SED)	Un	Pi	(SED)	Un	Pi	(SED)	Un	Pi	(SED)	Un	Pi	(SED)
Macroarthropods															
Total adult Staphylinidae	0.58	0.45	(0.04)*	0.66	0.41	(0.10)	0.63	0.43	(0.09)	0.56	0.40	(0.03)	0.54	0.40	(0.04)*
Corticariinae (Lathridiidae)	0.02	0	(0.02)	0.08	0.22	(0.02)**	0.07	0.20	(0.03)*	0.24	0.21	(0.02)	0.23	0.18	(0.04)
Aphididae	0.98	0.08	(0.03)***	1.03	0.54	(0.10)*	0.98	0.50	(0.05)**	0.24	0.13	(0.04)	0.06	0.03	(0.02)
Total Homoptera	1.13	0.41	(0.07)	1.24	0.95	(0.03)**	1.29	1.00	(0.05)*	0.68	0.55	(0.10)	0.55	0.41	(0.18)
Hymenoptera Parasitica	0.54	0.44	(0.10)	0.86	0.77	(0.07)	0.92	0.85	(0.02)*	0.79	0.68	(0.11)	0.75	0.59	(0.08)
Total macroarthropod catch	1.67	1.44	(0.06)*	1.92	1.78	(0.04)	1.89	1.74	(0.04)*	1.80	1.76	(0.05)	1.78	1.73	(0.05)

Asterisks show effects identified in analysis of variance (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$). (*) indicates an interaction between treatment and field but where the treatment effect was consistent and significant ($P < 0.05$) in three out of four fields. Summary data for taxa that did not exhibit significant effects of pirimicarb are given in Supplementary Table S3.

variance (36%; $P = 0.002$) and clearly illustrates significant negative effects of chlorpyrifos and cypermethrin at the macroarthropod community level. Inclusion of aphids would add one significant value of c_{dt} for pirimicarb to the PRC diagram (6d after treatment, $c_{dt} = -0.27$; $P = 0.03$) and the diagram would then display 43% of the treatment variance ($P = 0.002$). With the exception of Corticariinae, all taxa had positive species weights (Fig. 1b). The negative weight of Corticariinae reflects both a positive effect of pirimicarb (Table 3) and lack of an effect of chlorpyrifos (Table 1).

3.4. Responses of individual macroarthropod taxa

The null hypothesis that catches were independent of the chlorpyrifos treatment was rejected for 14 macroarthropod taxonomic groups (Table 1). Abundance of most macroarthropod taxa was reduced by chlorpyrifos, with Delphacidae (Homoptera), parasitic Hymenoptera and Diptera the groups affected most strongly. The total catch of macroarthropods and the overall taxonomic richness were reduced significantly by chlorpyrifos on all post-treatment sampling dates (Table 1).

For cypermethrin, the null hypothesis of no treatment effects was rejected for eight macroarthropod taxa (Table 2). With the exception of Thysanoptera (thrips), in all cases where effects of cypermethrin were statistically significant, the effects on abundance were negative. Thysanoptera were initially significantly increased in abundance by cypermethrin, followed by a significant decrease (Table 2). Increased abundance of thrips might reflect hormoligosis (stimulation of reproduction; Lucky, 1968) or effects on predators or competitors (other herbivores). Cypermethrin significantly reduced the total macroarthropod catch on three of the five post-treatment sampling dates (Table 2), but did not significantly affect the overall taxonomic richness (Supplementary Table S2).

Only six macroarthropod taxa exhibited effects of pirimicarb; there was no effect of pirimicarb on macroarthropod taxonomic richness (Table 3). The clearest effect of pirimicarb was on aphids, reflected also in the total Homoptera. The significant difference from unsprayed plots was transient, as a natural decline of aphid abundance occurred in all plots 20–30 days after insecticide application. Staphylinidae and parasitic Hymenoptera were also less numerous in pirimicarb-treated plots after the insecticide application, with the difference significant on one or two dates (Table 3), perhaps reflecting an indirect effect of aphid prey availability. Negative effects of pirimicarb were statistically significant for the overall macroarthropod catch on two of five post-treatment sampling dates (Table 3). However, these statistically significant effects of pirimicarb are marginal if Type I errors are taken into account (see Section 4).

3.5. Collembola-predator ratios

Collembola-predator ratios were calculated for Collembola species that exhibited positive effects of cypermethrin (*Entomobrya multifasciata*, *Sminthurinus aureus* group and *Sphaeridia pumilis*) and for macroarthropod taxa that are important

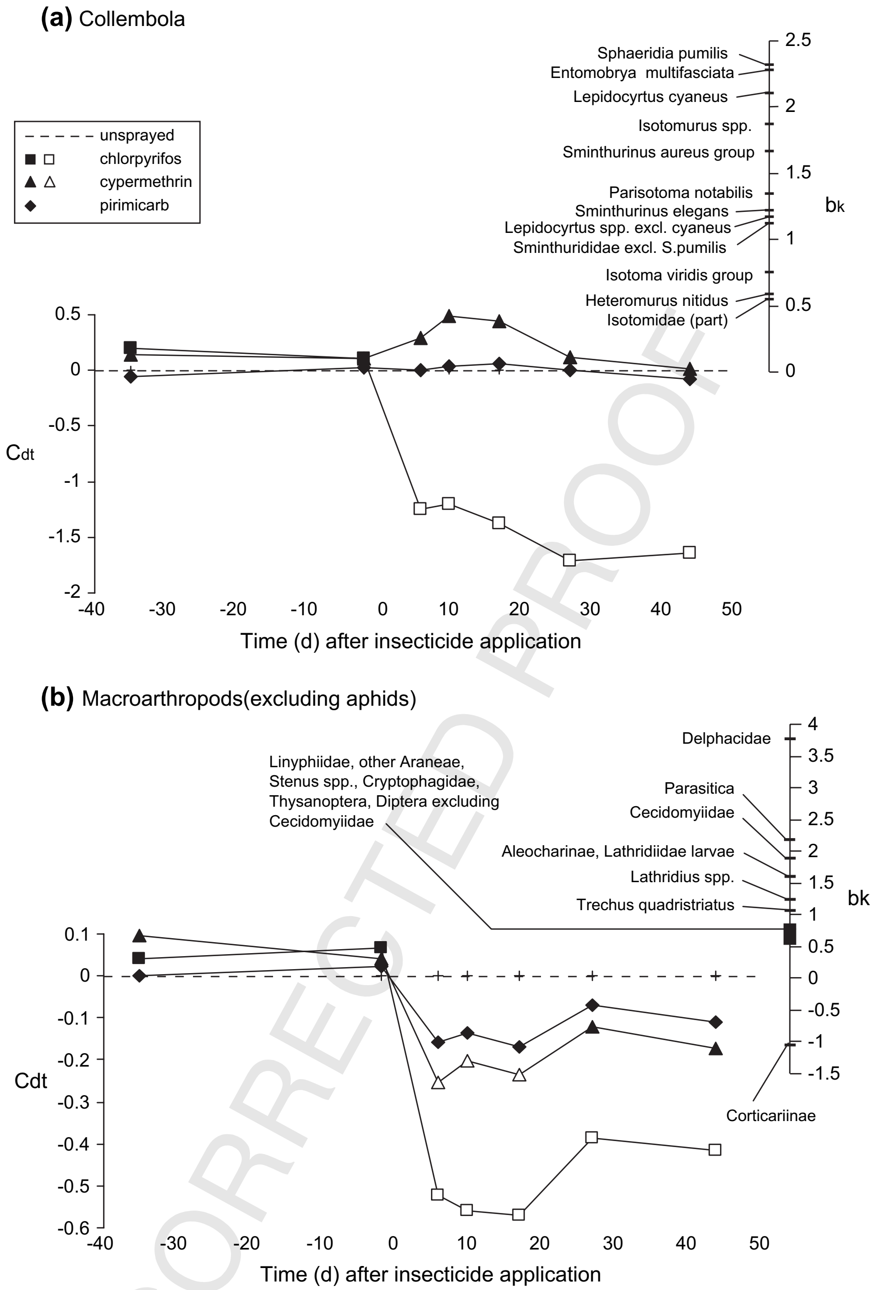

Fig. 1. Principal response curves (c_{dt}) and species weights (b_k) showing changes in the fitted abundance of (a) Collembola and (b) macroarthropods in plots sprayed with chlorpyrifos, cypermethrin and pirimicarb relative to unsprayed plots (reference treatment; $c_{dt} = 0$). White symbols indicate differences from the unsprayed control that are statistically significant for individual sampling dates ($P = 0.03$).

predators of Collembola (Carabidae, *Tachyporus* spp. larvae and Linyphiidae). The Carabidae data excluded herbivorous species and were dominated by *Trechus quadristriatus* (Appendix 1). Many predators of Collembola are generalists so it is not possible to elucidate precise predator-prey relationships; accordingly, only predators known to be important consumers of Collembola (e.g., Hopkin, 1997) were included. The log ratio was calculated using the means of log-transformed abundance for the three Collembola taxa combined, and for the three predator taxa combined. The ratio shows a clear difference between the treatments, being significantly higher (based on 95% CL) after the application of cypermethrin (Fig. 3).

4. Discussion

4.1. Generality of the findings

Effects of the insecticides on macroarthropods were largely as would be expected, with the most persistent effects observed for the broad-spectrum organophosphate chlorpyrifos and the most selective and transient effects for the narrow-

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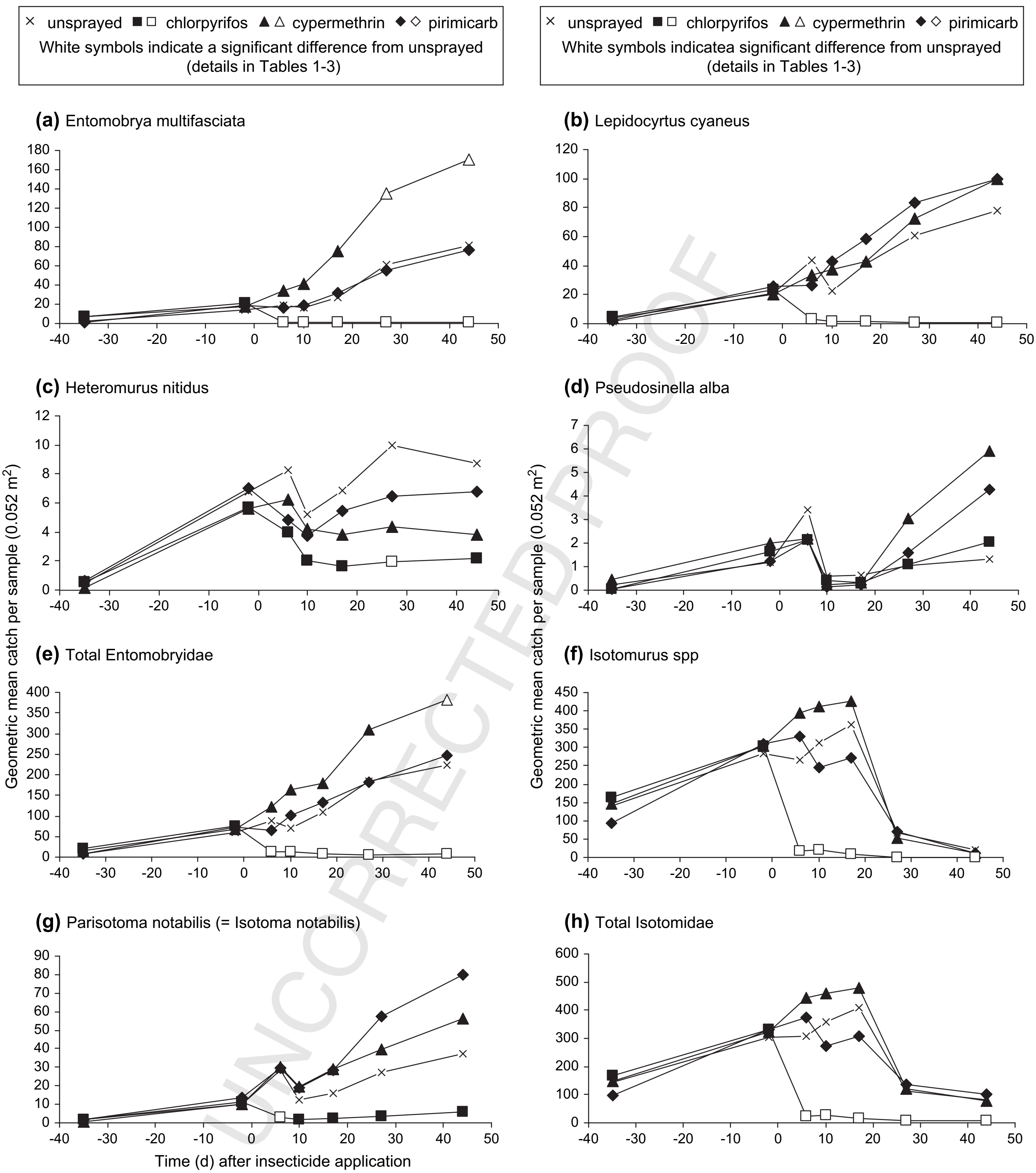

Fig. 2. Abundance of Collembola in plots sprayed with chlorpyrifos, cypermethrin and pirimicarb. Each data value is the geometric mean catch from 5 samples per treatment replicated in 4 fields ($n = 20$). Details of statistically significant effects (indicated by the white symbols) are given in Tables 1–3.

spectrum aphicide pirimicarb. Effects of chlorpyrifos on the epigeic Collembola community are also very similar to those observed previously (e.g., Frampton, 2002), suggesting that the exceptionally heavy rain that fell after insecticide applications did not substantially influence non-target effects. The taxonomic spectrum of effects of the insecticides on Collembola might have been underestimated, however, due to the patchy spatial distribution of some species. *Sminthurinus*

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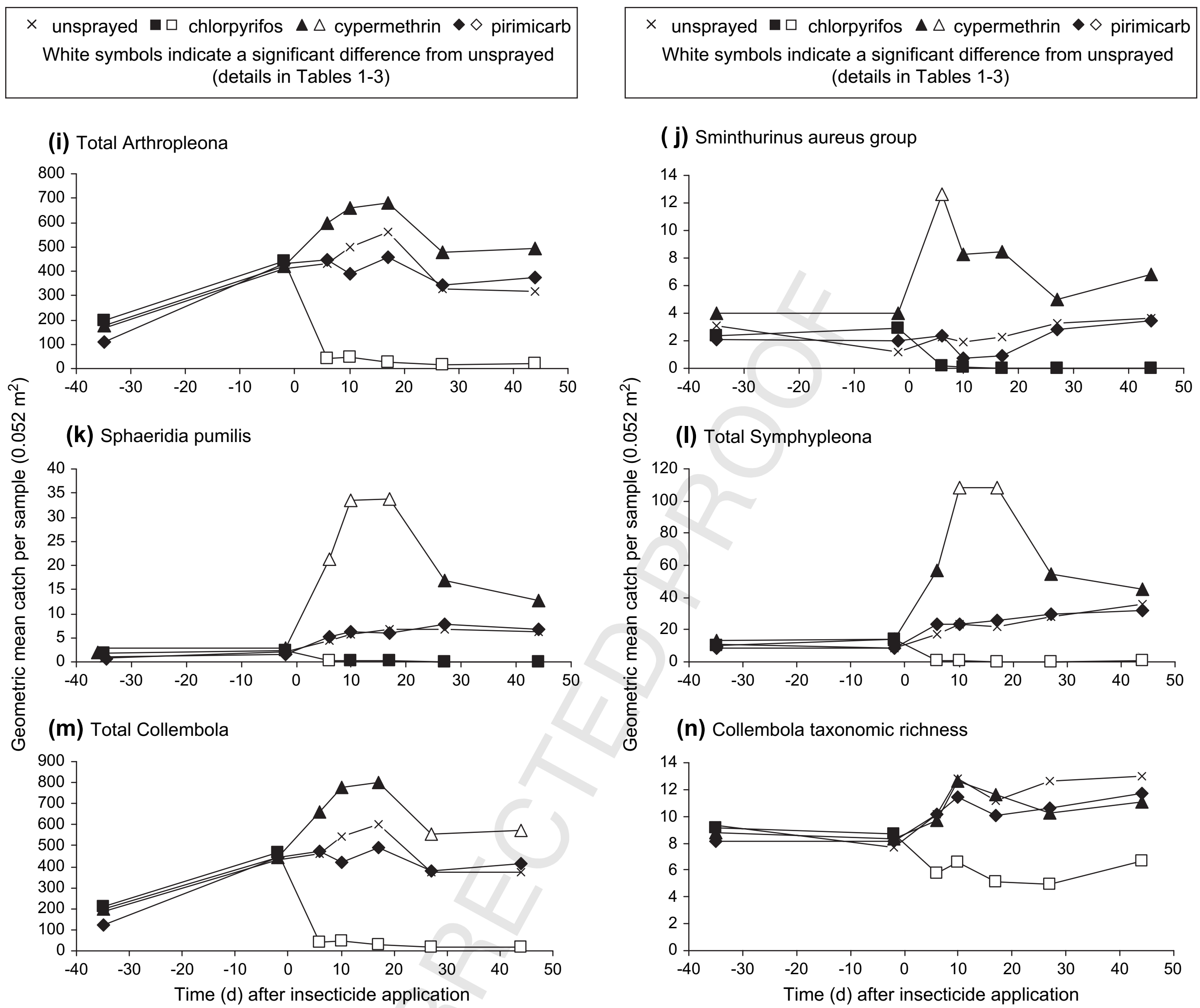

Fig. 2. *continued*

elegans, for example, increased markedly in abundance after cypermethrin treatment but on most sampling dates was almost entirely restricted in distribution to one field, precluding analysis. Effects of cypermethrin may also have been underestimated because *Entomobrya multifasciata* and *S. elegans* are often the dominant species in arable crops (e.g. Frampton, 2002; Frampton and van den Brink, 2002), but in this study were subordinate to a taxon unaffected by cypermethrin (*Isotomurus* spp.).

4.2. Data analysis

The taxonomic spectrum of effects is broadly consistent with the preliminary findings (Frampton, 1999). However, effects of pirimicarb on Collembola were not detected in the current work, whereas two suction-sampled Collembola taxa were affected significantly in the preliminary analysis (Frampton, 1999). The preliminary analysis was based on one pre-treatment and one post-treatment sampling to test the null hypothesis that the pre-to-post-treatment change in abundance between these samplings was independent of the pesticide treatment. In that analysis, the relevant factor was the interaction between treatment and time (Frampton, 1999). Such an analysis takes into account existing pre-treatment spatial variation, which might be important for changes in arthropod populations over relatively short timescales. However, the relevance of pre-treatment spatial heterogeneity over longer timescales is unclear and might not be consistent across species (as changes in abundance would be dependent on dispersal ability). As the current analysis is based on data for a longer time period, adjustment for pre-treatment abundance is not included. The current model also includes field as a random factor (cf. Frampton, 1999), as the study fields appear more typical a sample of wheat fields than was originally supposed.

For each taxon or PRC analysis, 21 statistical comparisons were carried out (7 dates × three insecticide comparisons

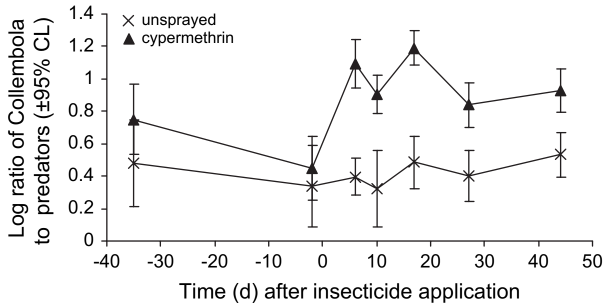

Fig. 3. Collembola-predator ratios in cypermethrin-treated and unsprayed plots based on the Collembola *Entomobrya multifasciata* + *Sminthurinus aureus* + *Sphaeridia pumilis* and the predators Carabidae + *Tachyporus* spp. larvae + Linyphiidae. Data are mean log ratios of Collembola to predators, based on 5 samples per treatment replicated in 4 fields ($n = 20$).

against the unsprayed treatment). With an experiment-wise error rate of $\alpha = 0.05$, approximately one significant effect per taxon or PRC diagram might have occurred by chance. A large number of taxonomic units (44) was also tested simultaneously on each of seven sampling dates in the univariate analyses, giving the possibility that for each individual pesticide as many as 15 of the significant effects might have occurred by chance. This would not affect the overall conclusions for chlorpyrifos and cypermethrin but suggests that for pirimicarb the effects were marginal and at the limits of statistical significance (only 12 of the tests were significant; Table 3). However, such strict interpretation of Type I error has been criticized, as $\alpha = 0.05$ gives a conservative estimate of Type I error in ecological field studies (Hinds, 1984).

4.3. Collembola-predator relationships

A plausible explanation for the increased catches of Collembola following the application of cypermethrin is that the insecticide had a greater negative effect on predators of Collembola than on the Collembola themselves, leading to a classical resurgence (Sheals, 1953; Hardin et al., 1995). As well as being directly toxic to predatory arthropods, cypermethrin may act as a feeding repellent and has been found to temporarily reduce prey consumption rates in spiders, independent of their abundance (Shaw et al., 2006). Predatory Acari, which were not monitored, are also important collembolan predators that are highly sensitive to synthetic pyrethroid insecticides. Although the Collembola-predator ratio is not proof of causality (Hardin et al., 1995), these findings suggest that the predation pressure on Collembola is unlikely to have been independent of the cypermethrin treatment.

As Collembola are preyed upon by both generalist and specialist predatory arthropods (Hopkin, 1997), it may appear surprising that the indirect effects of cypermethrin were taxonomically specific. One explanation might be that, due to spatial heterogeneity, not all effects of cypermethrin could be detected statistically (as with *S. elegans*; discussed above). Selective predation may have occurred in some cases, for example *Tachyporus* spp. larvae (Staphylinidae) in the samples were occasionally seen to have trapped Collembola with their mandibles and in all cases the captured Collembola were *Sminthurinus* spp. Furthermore, stenophagous carabid beetles may capture Collembola of particular size classes (e.g., Bauer, 1985).

4.4. Implications for risk assessment

The available information (the current work, together with four other published studies and three unpublished communications from contract testing laboratories cited above) suggests that Collembola resurgences may be relatively frequent when synthetic pyrethroid insecticides are used. Other broad-spectrum insecticide types also cause Collembola resurgences (e.g., Sheals, 1953) and resurgences may depend on the pesticide concentration (e.g., Funke et al., 1994). However, synthetic pyrethroids appear particularly likely to favour increased collembolan abundance due to the large difference in susceptibility of Collembola and their predators.

Collembola might be useful as indirect indicators of adverse pesticide effects on predatory arthropods, since the observed collembolan abundance integrates effects of pesticides on the full guild of Collembola-antagonists, including groups that are not routinely monitored in field studies (e.g. predatory Acari). The current study shows that monitoring the total collembolan abundance in the field cannot detect taxonomically specific effects, so focused monitoring of species that are representative, easily identified and responsive to the chemical treatments may be more appropriate. *Entomobrya multifasciata* is a potential candidate as an indicator in field studies, as it easily identified without the need for specialist taxonomic resources, it is widespread and often dominant in arable crops, and its numerical response detected both the negative effect of chlorpyrifos and the positive (indirect) effect of cypermethrin. The current study was, however, too short (up to 44 d after treatment) to determine the persistence of the indirect effects of cypermethrin on this species.

Current pesticide risk assessments for soil invertebrates in the EU involve routine testing of earthworms (*Eisenia fetida*). A recent review of pesticide effects on soil invertebrates recommended that Collembola (*Folsomia candida*) should also be tested routinely, as a representative of soil arthropods, because testing with oligochaetes alone does not identify all insecticide risks to soil invertebrates (Frampton et al., in press). The current work suggests that, for synthetic pyrethroids, a single-species Collembola test would be unlikely to identify risks to soil arthropods, as Collembola appear generally insensitive to these insecticides. There appears to be a case for the routine testing separately of Collembola and predatory soil arthropods, in addition to earthworms. A test for predatory soil arthropods (using the soil Acari *Hypoaspis aculeifer*) is currently under development (Bakker et al., 2003). Recent reviews of the effects of pesticides on soil invertebrates in laboratory studies (Frampton et al., in press) and field studies (Jänsch et al., in press) have confirmed that, except for earthworms, in most cases there is insufficient data from field studies to validate risk predictions that are based on laboratory testing. Chlorpyrifos is among the pesticides that have the best availability of field data for effects on soil invertebrates (Jänsch et al., in press) and the current work confirms well-known adverse effects of chlorpyrifos on Collembola. However, despite the widespread use of cypermethrin and pirimicarb, there is almost no information available in the open literature on the effects of these insecticides on Collembola in laboratory tests (Frampton et al., in press).

5. Conclusions

Cypermethrin and pirimicarb appear unlikely to pose risk to Collembola communities in arable agriculture at recommended application rates. However, resurgences of Collembola may be expected following some synthetic pyrethroid applications but the full taxonomic spectrum and persistence of such effects might have been underestimated in the current study. The taxonomic specificity of indirect effects on Collembola calls for a better understanding of soil invertebrate interactions to improve pesticide risk assessments, for example to clarify whether collembolan taxa might indicate adverse pesticide effects on other arthropods that are impractical to monitor routinely. Due to the taxonomic specificity of responses, monitoring only of total Collembola abundance or richness would not accurately estimate pesticide risks unless the aim is restricted to detecting general effects of broad-spectrum organophosphorus compounds.

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Hugh Lowe Farms Ltd. kindly supported the original field study with the provision of insecticides, manpower and machinery. The UK Department for Environment, Food and Rural Affairs (DEFRA) Pesticides Safety Directorate (PSD) provided funding to enable the processing and analysis of archived samples (Project PS2324).

Appendix 1

Taxonomic groups included in the analyses. Asterisks indicate groups with low abundance that were analysed using only the PRC approach. The principal trophic guilds are: F: fungivores; H: herbivores; O: omnivores; P: predators

Collembola: Arthropleona (F, O)

Entomobryidae
- *Entomobrya multifasciata*
- *Entomobrya nicoleti**
- *Lepidocyrtus cyaneus* group
- other *Lepidocyrtus* spp.
- *Orchesella cincta**
- *Orchesella villosa*
- *Heteromurus nitidus*
- *Pseudosinella alba*
- other *Pseudosinella* spp.*
- *Tomocerus* spp.*

Isotomidae
- *Isotoma viridis* group
- *Isotomurus* spp.
- *Parisotoma notabilis*

Hypogastruridae*

Collembola: Symphypleona (F, H, O)

Sminthuridae
- *Bourletiella hortensis**
- *Deuterosminthurus* spp.*
- *Sminthurinus elegans*
- *Sminthurinus aureus* group
- *Sminthurus viridis**

Sminthurididae
- *Sminthurides signatus**
- *Sphaeridia pumilis*
- *Stenacidia violacea**
- other Sminthurididae

Coleoptera: Carabidae (P)
- *Asaphidion flavipes**
- *Bembidion lampros**
- *Bembidion obtusum**
- *Demetrias* + *Dromius* spp.*
- *Loricera pilicornis**
- *Notiophilus biguttatus**
- *Trechus quadristriatus*
- Carabidae larvae*

Coleoptera: Staphylinidae (P, F, O)
- Aleocharinae
- *Philonthus* + *Quedius* spp.*
- *Stenus* spp.*
- *Tachyporus* spp.
- *Tachyporus* larvae

Coleoptera: Lathridiidae (F)
- Corticariinae
- Enicmus spp.
- *Lathridius* spp.
- *Stephostethus* spp.
- Lathridiidae larvae*

Other Coleoptera families (H, O)
- Chrysomelidae*
- Cryptophagidae*
- Coccinellidae*
- Curculionidae*
- Elateridae*
- Phalacridae*

Diptera (O)
- Cecidomyiidae
- Predatory Diptera*
- other Diptera

Hemiptera (H)
- Aphididae
- Cicadellidae*
- Delphacidae
- Heteroptera

Thysanoptera (H)

Hymenoptera: Parasitica (P)

Araneae (P)
- Linyphiidae
- other Araneae*

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.envpol.2006.08.038.

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