

Effects of Benzimidazole and Triazole Fungicide Use on Epigeic Species of Collembola in Wheat

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Foliar sprays of the systemic fungicides carbendazim, propiconazole, and triadimenol were applied in summer to replicated barrier-enclosed and open plots in a field of winter wheat in southern England at dose rates equivalent to label recommendations. Surface-active Collembola (springtails) were sampled from the experimental plots by suction sampling and pitfall trapping before and after the fungicide applications. No consistent effects of the fungicides on collembolan activity were detected using pitfall trapping but suction sampling revealed a transient negative effect of propiconazole and triadimenol on the overall abundance of higher collembolan taxa. Among individual species, however, fungicide effects varied spatially. Fewer significant treatment effects were obtained in enclosed than in open plots and no consistent effects of carbendazim were detected. The relevance of these findings to current fungicide usage strategies in British arable crops, which include the use of complex tank mixes, is discussed. © 2000 Academic Press

Key Words: side effects; sampling method; plot barriers.

INTRODUCTION

In Britain, wheat constitutes 41% of the area sown to arable crops and most (98%) of the ca. 1.9 million ha of wheat grown annually (ca. 9% of the total land area of Britain) are treated with fungicides. Wheat crops usually receive several applications within a season; in 1996, for example, there were on average four fungicide treatments per hectare (Thomas *et al.*, 1997). Collembola are among the most abundant arthropods inhabiting temperate farmland and may be exposed directly or indirectly to fungicide applications in spring and summer. They are widely preyed upon by other arthropods and are an important component of the arthropod food web. The majority of species are at least facultatively mycophagous and hence potentially at risk from indirect effects of fungicides on food availability.

However, in a review of side effects of pesticides on soil fauna, Edwards and Thompson (1973) could trace only one study of the responses of Collembola to fungicides. More recent work (Frampton, 1994; Hopkin, 1997) found that studies of fungicide effects on Collembola are less frequent than those of insecticides or herbicides and have not kept pace with increasing fungicide usage. Of 41 fungicide active ingredients applied to arable crops in Britain in 1996 (Thomas *et al.*, 1997), the effects of six (benomyl, carbendazim, cymoxanil, metalaxyl, propiconazole, and triadimenol) have been examined on Collembola (Frampton, 1988; Kiss and Bakonyi, 1990; Krogh, 1991; Kaluz *et al.*, 1993; Filser, 1994; Frampton, 1994), but only three studies were in the field. Pyrazophos has been the most intensively studied fungicide with regard to its effects on Collembola (Sotherton *et al.*, 1987; Frampton, 1988), largely because it is an organophosphate with broad-spectrum insecticidal activity. In Britain, however, pyrazophos is no longer used in arable crops (Thomas *et al.*, 1997) and has only minor usage on vegetable crops (Garthwaite *et al.*, 1997).

The sparsity of information on collembolan responses to fungicides prompted laboratory bioassays during the 1980s to ascertain whether selected chemicals are directly toxic. Residual exposure to carbendazim, propiconazole, pyrazophos, and triadimenol reduced the survival of *Sminthurinus aureus* (Lubbock) (Frampton, 1988), while fenarimol and propiconazole had high contact toxicity to *Folsomia candida* Willem (Kiss and Bakonyi, 1990). Adverse effects of pyrazophos on field-resident Collembola were subsequently confirmed in autumn-sown and spring-sown barley by Sotherton *et al.* (1987) and Frampton (1988) but to date no field studies of the effects of carbendazim, propiconazole, or triadimenol on Collembola have been published. These chemicals rank, respectively, at 2, 5, and 16 in the principal 50 pesticides used in Britain in 1986–1996 (Thomas and Wardman, 1999) and use of both carbendazim and triadimenol has recently increased (Fig. 1). Given the lack of any recent progress in field studies of the responses of Collembola to fungicide use, described here are field

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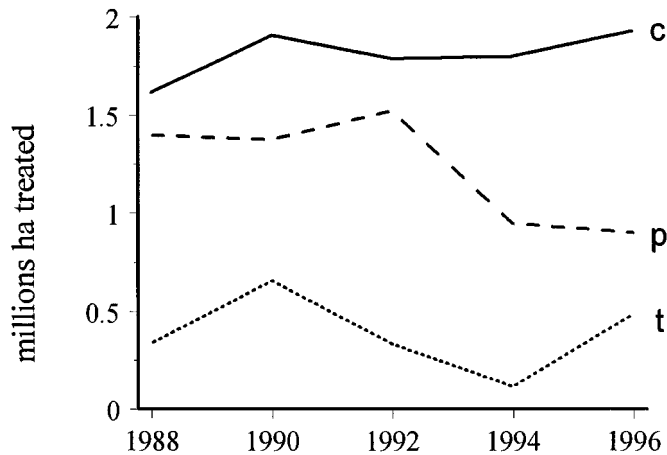


FIG. 1. Use of carbendazim (c), propiconazole (p), and triadimenol (t) in Britain, 1988–1996. From Davis *et al.* (1990, 1991, 1993), Garthwaite *et al.* (1995), and Thomas *et al.* (1997).

experiments that investigated the effects of these chemicals on Collembola in winter wheat during summer 1986.

MATERIALS AND METHODS

Study Site

The work was carried out in summer 1986 in a 19.0-ha field of winter wheat cv Mission on a commercial farm in Hampshire, southern England (51°3'N 1°30'W). The crop was drilled on 31 October 1985, following peas (1984), grass (1983), and barley (1982). Soil type was a light loam over chalk (Andover series). A preliminary assessment of Collembola numbers at the study site was obtained on 22 April when the crop and soil were dry using a randomly placed "D-vac" petrol-driven suction sampler (Dietrick Backpack Model 1A; Dietrick *et al.*, 1960). Each sample (0.46 m²) consisted of five pooled 0.092-m² subsamples obtained by holding the suction nozzle (diameter 0.342 m) on the ground for 10 s at each of five randomly chosen locations. The sampler nozzle contained a muslin net (mesh < 100 µm to ensure that Collembola nymphs were trapped and which was sufficiently long to permit crop plants to enter the net). Nine collembolan species were recorded, with mean densities ($n = 15$) up to 693 m⁻² (Frampton, 1989).

Experimental Design

Small plots were used to permit fungicide application using a knapsack sprayer. Two 10-m-spaced lines, each of 16 plots with each plot measuring 10 × 10 m, were marked out along a slope in the field (Fig. 2). Plots were more than 150 m from the nearest field boundary. As a contingency against arthropod redistribution by dispersal across the relatively small plots, those in one of the lines were each

enclosed by polythene exclusion barriers dug 20 cm into the ground and extending 30 cm above it. Such barriers impede the movement of surface-dwelling Collembola (Gravesen and Toft, 1987). Plots in the remaining line were left open. Barrier-enclosed plots were 2 m apart, whereas a greater (5 m) separation of open plots was used to reduce the likelihood of arthropod dispersal between adjacent plots. The barriers were inserted into the ground on 1 May with a tractor-drawn cloche plough and were subsequently raised and supported with rope and posts on 3 May. A linear arrangement of plots was used for two reasons: (1) disruption of routine farm operations in the field was minimized by accommodating all the plots between adjacent sets of tractor wheelings; and (2) this design permitted the use of a plough to ensure that all plot barriers were constructed simultaneously. Because barrier-enclosed and open plots were not randomized, analyses were performed separately for each of these plot types, which effectively constituted two experiments (but with similar null hypotheses; see below). The use of 16 plots permitted potential variation in arthropod abundance with elevation to be partitioned in the data analysis using a linear arrangement of four blocks, each of which contained one replicate of each treatment (Fig. 2).

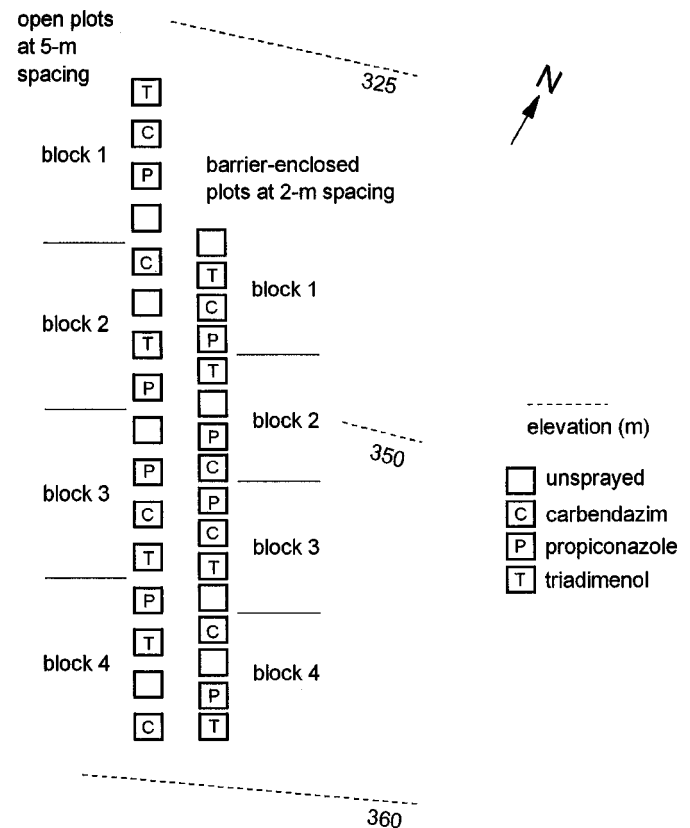


FIG. 2. Layout of the experimental plots (not to scale).

Fungicide Applications

The fungicides used were the benzimidazole carbendazim (Bavistin FL, BASF, 50% ai SC) and the triazoles propiconazole (Tilt 250EC, Ciba-Geigy, 25% ai EC) and triadimenol (Bayfidan, Bayer, 25% ai EC). These chemicals were widely used on U.K. arable crops (Fig. 1) and all significantly reduced survival of a collembolan test species (*S. aureus*) in laboratory bioassays (Frampton, 1988). Product label recommended application rates for these fungicides were 0.5 liter ha⁻¹ product in a minimum of 200 liters ha⁻¹ water.

Fungicides were applied to the experimental plots using a Cooper Pegler CP3 Mk. 220-liter-capacity knapsack sprayer. A 1.0-m spray boom fitted with three 80° flat fan nozzles (type 00) 0.5 m apart gave an effective swath width of 1.5 m at the recommended boom height of ca 0.6 m above the crop (British Crop Protection Council, 1986). For convenience a standard volume rate of 240 liters ha⁻¹ was used for each fungicide, obtained with a sprayer output between 3.6 and 3.9 liters min⁻¹ at a forward speed of ca. 6.2 km h⁻¹ and tank pressure of ca. 2.0 bar. A 20-liter tank load was sufficient to spray the eight replicate plots (four barriered and four open) for each treatment. The sprayer was cleaned thoroughly after each fungicide application by flushing with at least 80 liters of water. Spraying was performed under dry conditions with surface wind speed between 3 and 6 km h⁻¹ to minimize risk of drift (British Crop Protection Council, 1986). Control plots were left unsprayed.

Fungicides were sprayed on 12 June (growth stage 30–39; Tottman, 1987). A second application of each fungicide was made only to the open plots on 17 June (growth stage 39–45), following product label recommendations. These application times were realistic, as they concurred with an increase in the incidence of fungal pathogens at the study site (powdery mildew, *Erysiphe graminis* DC.; eyespot *Pseudocercospora herpotrichoides* Deighton; and leaf spot *Septoria* spp.) during the first 2 weeks of June. Carbendazim is recommended for use against eyespot and *Septoria* spp. up to growth stage 59, propiconazole against powdery mildew up to growth stage 51, and triadimenol against powdery mildew up to growth stage 71.

Pesticides other than the test fungicides were not applied during the study but all plots received inorganic nitrogen in February (47 kg ha⁻¹), April, and May (60 kg ha⁻¹), and sulfur as a foliar feed on 10 June (8 kg ai ha⁻¹).

Sampling Methods

The number of samples needed to estimate accurately mean abundance (suction samples) or activity (pitfall captures) of *Collembola* with a known precision (S.E. ≤ 20% of the mean) was determined using a power-law relationship between the sample variance and mean (Finch *et al.*, 1978). Three suction samples and 10 pitfall traps per plot were

chosen (Frampton, 1989; summarized in Frampton, 1994); this was a compromise between sampling precision (many samples desirable) and the potentially high sample processing effort and risk of population depletion (few samples preferable).

Suction samples were collected only when the crop and soil were dry. Arthropods were transferred from the sampler net into polyethene bags when the sampler engine was idling; samples were subsequently placed in 70% methyl alcohol within 4 h of collection for storage. On each sampling occasion, three D-vac samples were taken randomly within each plot, avoiding the area within 1 m of the plot edge. Blocks were sampled in a different sequence on each sampling date and sampling was completed within 3 h. The efficiency of the D-vac at removing epigeic *Collembola* from the soil surface was in the range 97–100% for the eight species examined (Frampton, 1989).

Each pitfall trap consisted of a 7-cm-diameter white plastic beaker set in the soil with its rim level with the soil surface. In the center of each plot, 10 traps were arranged in two rows of five, 6 m apart, and with 1.5-m intervals between adjacent traps. This was to minimize disturbance to the crop during trap emptying and replacement. Traps each contained 100 ml of a weak detergent solution to ensure that the captured arthropods sank, hence avoiding damage due to dehydration and predation. No preservative was used during trapping. Traps were set for periods of either 4 or 7 days; arthropods were removed by sieving (mesh 150 µm) and stored in the same manner as suction samples. Suction and pitfall trap samples were collected on several occasions, up to 9 days pre- and 47 days post-treatment (Table 1).

TABLE 1
Timing of Fungicide Applications and Arthropod Sampling

| Date | Growth stage (Tottman, 1987) | Enclosed plots | Open plots |
|---------------|---------------------------------|--------------------|--------------------------|
| Pretreatment | | | |
| 3 June (–9) | 30–39 | P1 installed | P1 installed |
| 6 June (–6) | | S1 | S1 |
| 10 June (–2) | | P1 removed | P1 removed |
| 12 June (0) | | Fungicides sprayed | Fungicides sprayed |
| Posttreatment | | | |
| 13 June (+1) | 37–43 | P2 installed | P2 installed |
| 17 June (+5) | 39–45 | S2 | S2 |
| 19 June (+7) | | | P2 removed |
| | | | Fungicides sprayed again |
| 20 June (+8) | 41–59 | P2 removed | |
| 22 June (+10) | | P3 installed | P3 installed |
| 28 June (+16) | 55–65 | | P3 removed, S3 |
| 29 June (+17) | | P3 removed, S3 | |
| 22 July (+40) | >75 | P4 installed | P4 installed |
| 29 July (+47) | | P4 removed | P4 removed |

Note. P, pitfall traps; S, suction samples.

Most Collembola were identified according to Christiansen and Bellinger (1998) and Fjellberg (1980), which refer to Nearctic and Norwegian faunas, respectively, but include species of wider Holarctic distribution; identification of *Jeannenotia stachi* (Jeannenot) (= *Stenacidia violacea* Reuter *sensu* Christiansen and Bellinger, 1998) followed Betsch and Massoud (1970). Other European identification keys covering the British fauna exist but are now considered obsolete (Rusek, 1995).

Data Analysis

The principal aim of this work was to investigate effects of the fungicide treatments. Blocks and plot types (open or enclosed) were included as a contingency against arthropod spatial heterogeneity and potential redistribution across plots, but their potential influence on Collembola captures was not of primary interest. Plot type (open or enclosed) was confounded with position in the field (Fig. 2) and also with the number of fungicide applications (Table 1), and hence analysis of treatment effects was done separately for open and enclosed plots. Pitfall trap captures (which estimate a combination of activity and density) and suction catches (estimates of density) are not additive, so separate analyses were also carried out for each sampling method.

For each plot type and sampling method, the null hypotheses that mean Collembola captures did not differ between fungicide treatments (fixed factor *A* with four levels, *i*) and that treatment was independent of block (random factor *B* with four levels, *j*) were tested using the analysis of variance (ANOVA) model

$$X_{ijk} = \mu + A_i + B_j + AB_{ij} + e_{k(ij)},$$

where μ represents the mean of all the populations being sampled, X_{ijk} is the *k*th replicate in the combination of the *i*th level of fungicide treatment and the *j*th level of block, and $e_{k(ij)}$ is the residual error term (each replicate *k* is nested in the combination of fungicide treatment and block). Before analysis, Collembola counts *x* were transformed to $\log_{10}(x + 1)$ to remove departures from normality (confirmed with Kolmogorov–Smirnov normal probability plots for treatments pooled across blocks and *vice versa*). Significant heterogeneity of variances was not detected using Bartlett's test (Sokal and Rohlf, 1995), despite the high sensitivity of the test (Underwood, 1997). Where ANOVA revealed a significant interaction between treatment and block and also a significant treatment effect ($P < 0.05$), data were plotted graphically to visualize the nature of the interaction. Dunnett's (*a posteriori*) multiple contrasts (Sokal and Rohlf, 1995) were used to test for significant differences between each of the three fungicide treatment means and the control mean within each level of block (Underwood, 1997), after adjustment of the experiment-wise error rate (Dunn-

Šídák method; Sokal and Rohlf, 1995). Nonsignificant treatment effects in the ANOVA ($P \geq 0.05$) were not examined further, irrespective of any significant interactions with block.

RESULTS

Approximately 79,600 Collembola were captured, comprising 13 lower taxa (species or genera): *Sminthurus viridis* (L.), *Sminthurinus elegans* (Fitch), *S. aureus*, *Bourletiella hortensis* (Fitch), *Deuterosminthurus* spp., *J. stachi*, *Isotoma viridis* Bourlet, *Pseudosinella alba* (Packard), *Lepidocyrtus cyaneus* Tullberg, *Entomobrya multifasciata* (Tullberg), *Orchesella villosa* (Geoffroy), *Tomocerus longicornis* (Müller), and *Tomocerus minor* (Lubbock). The latter four species occurred only sporadically and at low abundance and were not included in the data analyses. For both plot types and sampling methods, the catch was dominated by *L. cyaneus* and *S. elegans* (Fig. 3). The abundance of two species clearly varied with distance along the height gradient: *P. alba* decreased in abundance from block 1 to block 4 (Figs. 4c and 5c), whereas *B. hortensis* was confined mainly to block 1. Collembola numbers declined markedly during June (Fig. 3) so that subsequently low counts precluded a meaningful analysis of treatment effects for *B. hortensis*, *J. stachi*, and *S. aureus*.

Pretreatment sampling in early June (Table 1) yielded no significant differences between treatments or blocks, for either sampling method or plot type (all ANOVA *F* ratios ≤ 3.86 ; $P \geq 0.05$). Accordingly, no adjustment of Collembola captures for pretreatment distribution was made when post-treatment analyses were carried out.

For pitfall samples, the null hypothesis that mean post-treatment Collembola captures did not differ between fungicide treatments was not rejected ($F_{3,9} \leq 3.86$; $P \geq 0.05$) for any collembolan taxon, in either open or enclosed plots. Graphical visualization of the data for each species (plotting treatment means within and across blocks) did not reveal any consistent patterns in pitfall trap captures between the fungicide treatments on any of the sampling dates.

Suction captures of seven collembolan taxa (*S. viridis*, *I. viridis*, *P. alba*, *L. cyaneus*, total Symphypleona, total Arthropleona, and total Collembola) exhibited significant differences between fungicide treatments 5 days after treatment ($F_{3,9} > 3.86$), but in all cases treatment \times block interactions were also significant ($F_{9,32} \geq 3.86$; $P < 0.05$). Graphical visualization of the data from open plots indicated that for these taxa the differences between mean control and fungicide-treated captures were nevertheless usually consistent in direction for propiconazole (Fig. 4) and triadimenol (Fig. 5). Among barrier-enclosed plots, however, only differences between control and propiconazole-treated plots for three taxa were consistent across all blocks (Fig. 6). Results of within-block pairwise contrasts between control and fungicide treatments (Figs. 4–6) supported the general conclusion

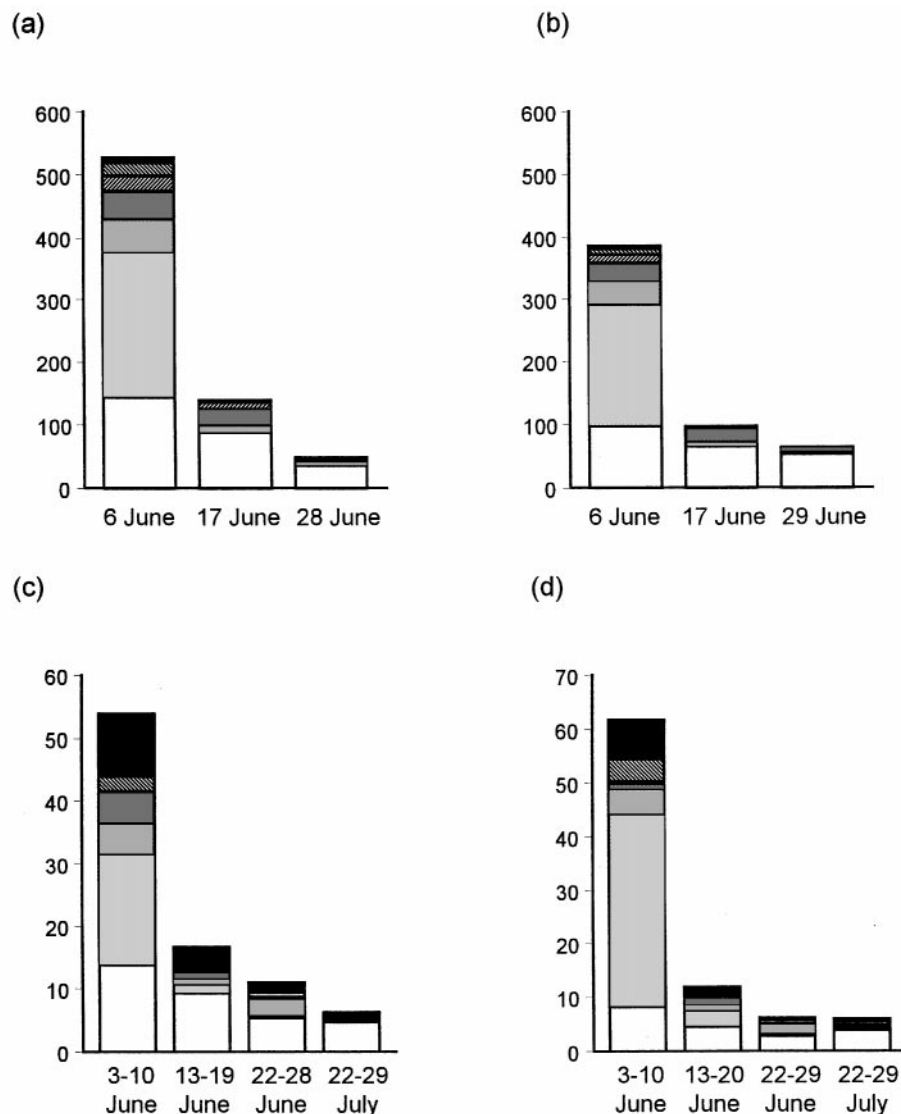


FIG. 3. Comparison of Collembola samples in control (unsprayed) plots (mean catches 0.46 m^{-2} suction sample ($n = 12$) or 7-day pitfall trap ($n = 40$)). (a) suction samples, open plots; (b) suction samples, barrier-enclosed plots; (c) pitfall trap samples, open plots; (d) pitfall trap samples, barrier-enclosed plots. Bh—*Bourletiella hortensis*; Ds—*Deuterosminthurus* spp.; Iv—*Isotoma viridis*; Js—*Jeannenotia stachi*; Lc—*Lepidocyrtus cyaneus*; Pa—*Pseudosinella alba*; Sa—*Sminthurinus aureus*; Se—*S. elegans*; Sv—*Sminthurus viridis*.

that overall effects of propiconazole and triadimenol on Collembola captures 5 days after treatment were negative but spatially variable. No significant treatment or treatment \times block effects were detected among suction captures of Collembola on subsequent sampling occasions, despite a second application of the fungicides to open plots (Table 1).

DISCUSSION

A substantial decrease in overall abundance (suction sampling) and in activity-density of Collembola (pitfall trapping) took place during this study. This limited the detection of effects of the fungicide treatments among *B.*

hortensis, *J. stachi*, and *S. aureus*, which were numerous only in pretreatment samples. The use of sulfur on 10 June as a foliar feed may have contributed to this decline; sulfur has broad-spectrum fungicidal activity (hence potential for influencing collembolan food availability) and is directly harmful to some beneficial insects (Hassan *et al.*, 1994, 1998). However, no direct effects of sulfur on Collembola have been reported and routine application of sulfur to all plots in another field trial (Frampton, 1988) did not influence overall collembolan abundance. Whatever its cause, the overall decline in collembolan abundance is part of the agricultural realism of this study; sulfur is used widely in cereals (e.g., Thomas *et al.*, 1997) and it is not unusual for

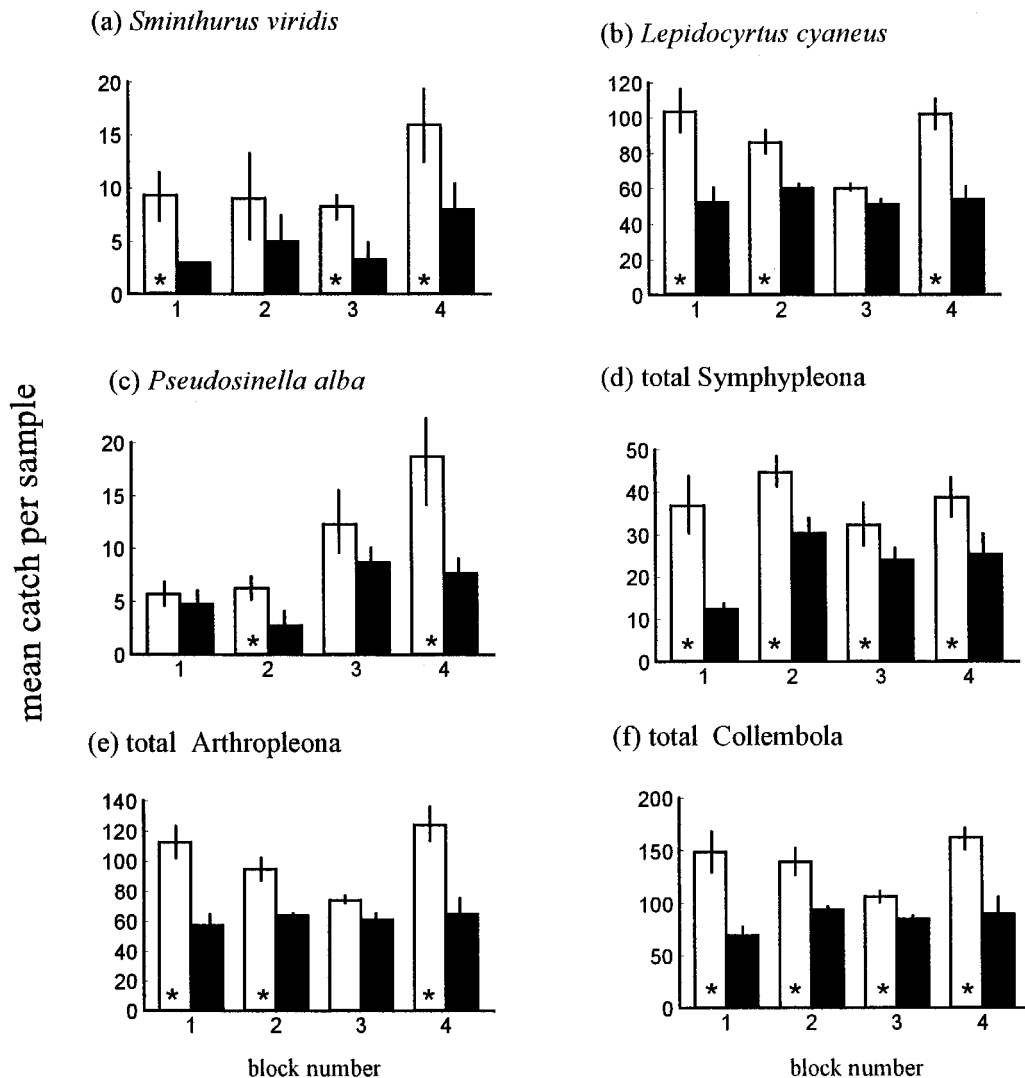


FIG. 4. Mean ($\pm 95\%$ CL, $n = 3$) suction samples of Collembola 0.46 m^{-2} in open control (unsprayed) (\square) and propiconazole-treated plots (\blacksquare) 5 days after treatment. For each pair of means, asterisks denote a significant difference (Dunnett's contrasts, $P \leq 0.013$).

summer peaks in the abundance of arable Collembola to be followed by abrupt declines during early June (Frampton, 2000).

Cereal fungicides are used from early April to July in the United Kingdom, so the current work could not address effects of fungicides at times of higher collembolan abundance earlier in the season. Captures of seven species that occur frequently in farmland were too low during the latter part of this study for analysis. One of these, *S. aureus*, exhibited increased mortality when exposed to residues of all three fungicides in laboratory bioassays (Frampton, 1988) but the field relevance of this needs to be established.

The results demonstrated negative effects of propiconazole and triadimenol, but not of carbendazim, on collembolan abundance in the field. Where differences be-

tween control and fungicide treatments were statistically significant, they were consistent in direction, with control numbers exceeding those from fungicide treatments. The lack of effects of carbendazim are, however, inconsistent with results from laboratory bioassays in which effects of carbendazim and propiconazole on mortality of *S. aureus* were similar (Frampton, 1988). Field effects may have been indirect, via fungi, whereas in the laboratory only direct effects were investigated. Prediction of field effects from laboratory results is complicated by the fact that Collembola exhibit feeding preferences among fungal species (Chen *et al.*, 1995; Hedlund *et al.*, 1995), while carbendazim and propiconazole are toxic to different species of fungi. The importance of indirect fungicide effects on the diet of fungivores has been indicated for Acari (Mueller *et al.*, 1990).

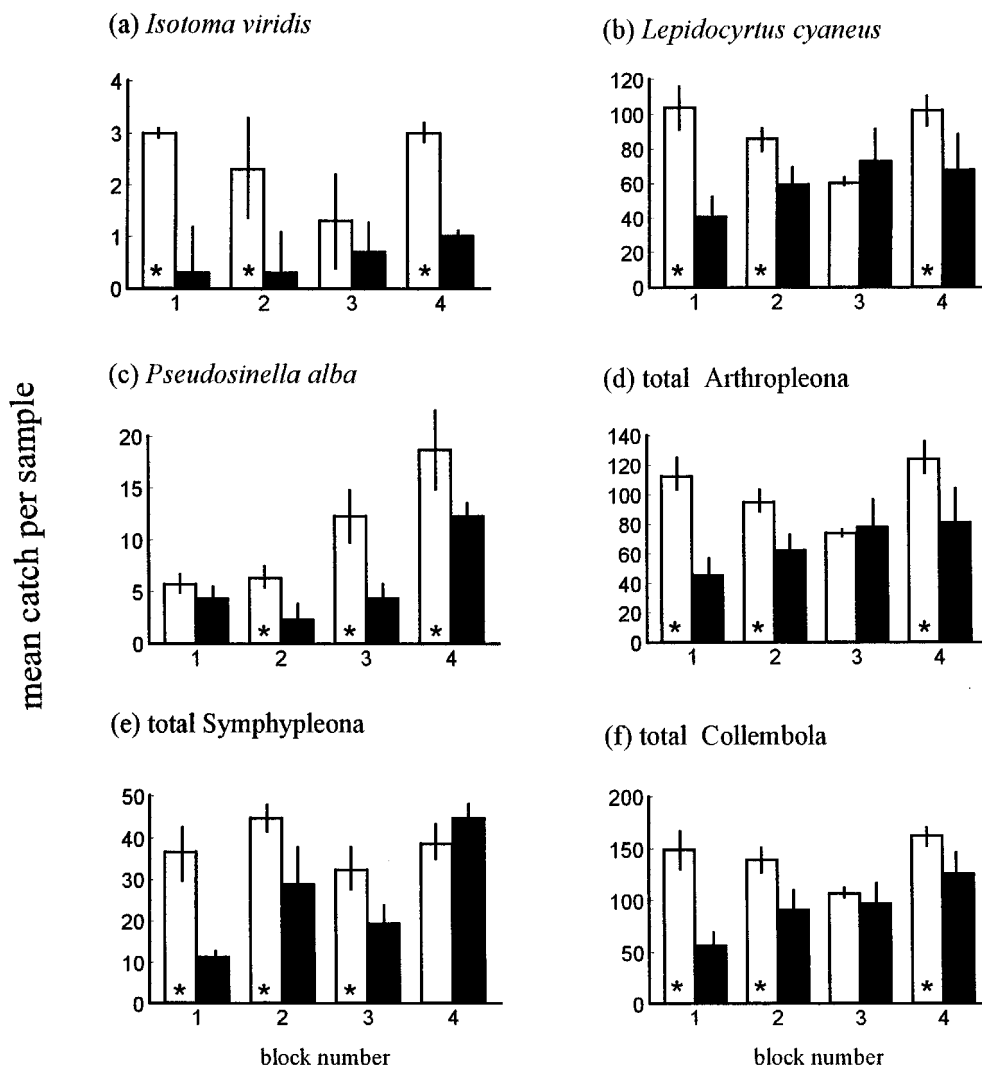


FIG. 5. Mean (\pm 95% CL, $n = 3$) suction samples of Collembola 0.46 m^{-2} in open control (unsprayed) (\square) and triadimenol-treated plots (\blacksquare) 5 days after treatment. For each pair of means, asterisks denote a significant difference (Dunnett's contrasts, $P \leq 0.013$).

and potentially complex interactions between fungicides, fungi, mycophages, and predators could occur where facultatively fungivorous predators prey upon Collembola (e.g., among Staphylinidae; Dennis *et al.*, 1991).

Three aspects of fungicide use make the generalization of results from any one ecotoxicological study difficult: (1) Products may contain more than one fungicidal active ingredient; (2) combinations of fungicide products may be applied simultaneously and with other pesticides in complex tank mixes; and (3) products (and mixes) may be applied more than once within a season.

In the present study, carbendazim, propiconazole, and triadimenol were applied as single active ingredients but in a recent survey two-thirds of fungicide products applied to British arable crops contained more than one active ingredient (Thomas *et al.*, 1997). During the past decade

there has been a shift from single product applications toward increasingly complex tank mixes containing up to five products (Thomas and Wardman, 1999). In many cases, use of multiple active ingredients within products, multiple products within tank mixes, and repeat applications of products or mixes has led to the use of smaller quantities of individual active ingredients. It does not necessarily follow, therefore, that multiple usage of a toxic chemical would lead to greater vulnerability of Collembola or other arthropods in the field. These factors illustrate the ecotoxicological complexity of evaluating fungicides against nontarget organisms.

In the current study, both open and barrier-enclosed plots had initially received identical fungicide treatments so that comparison of treatment effects between the plot types before 17 June is valid (Table 1). The relative lack of

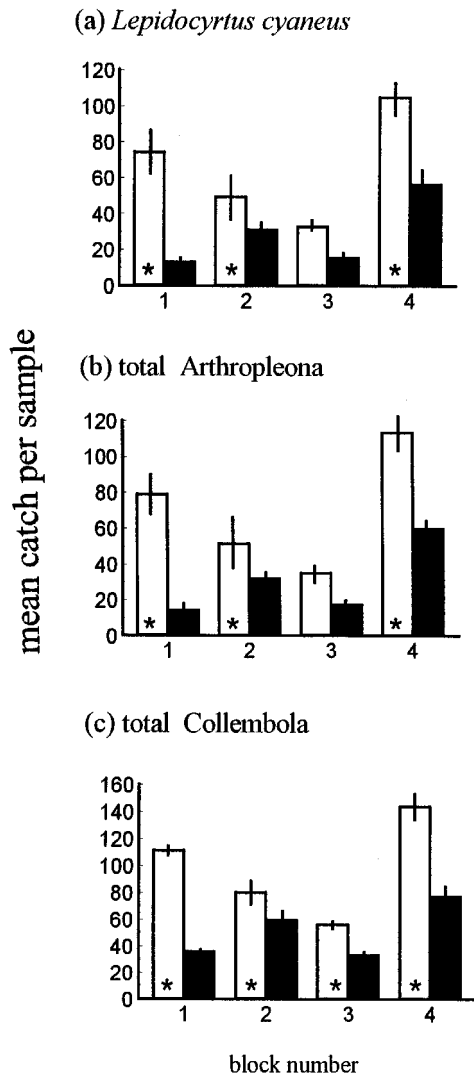


FIG. 6. Mean ($\pm 95\%$ CL, $n = 3$) suction samples of Collembola 0.46 m^{-2} in barrier-enclosed control (unsprayed) (\square) and propiconazole-treated plots (\blacksquare) 5 days after treatment. For each pair of means, asterisks denote a significant difference (Dunnnett's contrasts, $P \leq 0.013$).

consistent effects of the fungicide treatments in the barrier-enclosed plots suggests that barriers may actually be undesirable in some field trials with Collembola, which is reinforced by the fact that they are labor-intensive to construct and maintain (Davidson *et al.*, 1999). The ability to detect pesticide effects on Collembola in small ($< 10 \times 10 \text{ m}$) plots (Joy and Chakravorty, 1991; Krogh, 1991; Huusela-Veistola *et al.*, 1994) has advantages for experimental design where land availability is limited. However, such small plot sizes would be unsuitable for the study of pesticide effects on more highly dispersive predatory arthropods (e.g., Duffield *et al.*, 1996). The spatial variation (between blocks) in fungicide effects in the present work underlines the need to adequately replicate pesticide treatments in field trials and

test for interactions between treatment and spatial location (Underwood, 1997).

CONCLUSIONS

Most of the fungicides routinely applied to arable crops have not been evaluated for their safety to Collembola, despite the potential for indirect effects via changes in the availability of fungi in the collembolan diet. Negative effects of propiconazole and triadimenol were detectable at a time of declining collembolan abundance in the field, and relatively few species were available for this study. Further investigation of side effects of these chemicals on different species and at higher populations is advised. Considering the high sampling and sample-sorting effort involved in most field studies with arthropods, there appear to be no practical advantages in using barrier-enclosed plots or pit-fall sampling when suction sampling in open plots is an available option for studying epigeic species of Collembola.

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