Aspects of Applied Biology 37, 1994
Sampling to Make Decisions

Sampling to detect effects of pesticides on epigeal Collembola (springtails)

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SUMMARY

Results from recent European field studies investigating side-effects of pesticide use on epigeal Collembola are reviewed. *Sminthurus viridis, Sminthurinus elegans, Lepidocyrtus* spp., and *Isotoma viridis* could be useful as indicators of current pesticide use but it is not yet possible to use species or groups of Collembola as model predictors of pesticide effects. Consideration is given to sampling methods, the number and timing of samples, and the advantages and disadvantages of different experimental approaches for studying side-effects of pesticide use on epigeal Collembola.

INTRODUCTION

Except for Onychiuridae, which may be a target for insecticides in sugar beet, Collembola are regarded as non-target arthropods in field crops in northern Europe. Besides being potentially important in nutrient cycling (e.g. Richards, 1974), they are prey for farmland invertebrates including Carabidae (e.g. Sunderland, 1975) and Linyphiidae (Sunderland, 1986), and they could be important when other prey types, including pests, are scarce (Burn, 1992).

Studies of pesticide side-effects on Collembola and other invertebrates in the 1960s have been reviewed (Edwards & Thompson, 1973 *inter alia*) but some pesticides known to adversely affect them (such as organochlorines) are no longer approved for use. There have since been relatively few european studies of pesticide effects on Collembola in farmland but some currently-used chemicals are detrimental to epigeal species (Gregoire-Wibo, 1983; Sotherton, Moreby & Langley, 1987; Frampton, 1988; Frampton, 1989; Frampton & Çilgi, 1992; Vickerman, 1992; Filser, 1993a; Filser & Nagel, 1993; Filser *et al.*, 1994). Favourable effects of pesticides on some groups of Collembola have been reported (e.g. Frampton *et al.*, 1992), but this paper considers adverse effects. Collembola have potential as indicators of current pesticide side-effects (e.g. Heimann-Detlefsen, 1991; Filser, 1992; Vickerman, 1992; Filser, 1993b; Filser *et al.*, 1994) but no guidelines exist for the field assessment of pesticide side effects on these arthropods. This paper provides information on vulnerable species, sampling approaches and experimental designs for the assessment of pesticide effects on epigeal species.

MATERIALS AND METHODS

Results of recent work on epigeal Collembola in European farmland were surveyed to determine: the pesticides and species that have been studied; the species that are affected adversely by currently-used pesticides; whether particular species can be used as indicators of pesticide use; and whether it is important to monitor individual species. Information from recent studies was also used to recommend an appropriate standard sampling method, to identify ways of improving future studies, and to highlight potential problems in designing field experiments.

RESULTS

Pesticides studied

Recent field studies have shown that currently-used pesticides can adversely affect species or groups of epigeal Collembola (Table 1). Interpretation of the results of these studies should be cautious because: (1) the extent of available information varies considerably between species (some were trapped more frequently than others); (2) published work sometimes states only those pesticide effects that are obvious and/or statistically significant; this would give an under-representation of tolerant species or of relatively innocuous pesticides; (3) unlike some laboratory testing programmes (e.g. Riepert, 1993) there are no set guidelines for deciding what constitutes a pesticide effect in the field, so results given in Table 1 are necessarily based on opinions of the individual researchers; (4) the published studies differed in their aims and experimental protocols, such as in the timing, cropping, scale and location of experiments.

Most of the reported adverse effects of pesticides on Collembola concern fungicides and organophosphorus insecticides; other pesticide types have been relatively poorly studied (Table 1). Even synthetic pyrethroids and pirimicarb, which are among the most widely used pesticides in the UK (Davis *et al.*, 1991) have received little attention with regard to their effects on Collembola. At present there is insufficient field data to determine whether synthetic pyrethroids are likely to be generally harmful or not; the adverse effect of lambda-cyhalothrin (Table 1; Filser, 1992) was detected in hops in an unreplicated experiment of short duration and other data (U. Heimbach, unpublished) suggested that synthetic pyrethroids (fenvalerate and lambda-cyhalothrin) were not obviously detrimental to epigeal species in wheat. Everts *et al.* (1991) did not study side-effects but concluded that the majority of Collembola in oilseed rape plots would not have been exposed to a deltamethrin spray. The apparent adverse effect of pirimicarb on Collembola (in sugar beet) (Table 1; Büchs, 1991) should be interpreted with caution as the aim of that study was not to examine effects of individual pesticides; the effect of pirimicarb was, however, considered to be real (W. Büchs, personal communication).

Collembola as indicators

Indicators of pesticide effects fall into two categories; those that indicate the occurrence of side-effects and those that can be used as models to predict effects more widely, on other species or at the community level (see Çilgi, this volume). The studies summarised (Table 1) show that at least 12 species are adversely affected by currently-used pesticides, indicating that epigeal Collembola could be useful as indicators of off-target pesticide effects. They may be more sensitive than Coleoptera and Linyphiidae to summer applications of dimethoate (Frampton & Çilgi, 1993), and are known to be adversely affected by permanent cropping systems with intensive pesticide inputs such as hops, vineyards (Filser, 1993b; Filser *et al.*, 1994) and cereals (Vickerman, 1992). Negative effects on Collembola of intensive management can last for years, even after the management practice is converted into a less intensive form (Filser *et al.*, 1994; G.P.Vickerman, unpublished data) and although the total number may recover relatively quickly, the community structure may remain distinctly altered (Filser *et al.*, 1994). For this and other reasons it is not possible to select individual "model" indicator species for predicting effects at the community level, although as indicators of actual pesticide side-effects Collembola are potentially valuable. Here the results of recent work are used to determine whether it is important to monitor epigeal Collembola as a group, or whether it is acceptable to monitor one or a few individual species to detect pesticide effects.

Table 1. *Pesticides reported to have adverse effects on individual species and groups of epigeal Collembola; (i) insecticides; (f) fungicides; (m) molluscicides. Numbers refer to references in Table 2. Asterisks denote pesticides approved for use in EC but not UK crops.*

Species or group	Adverse effect seen	No adverse effect seen
Deuterosminthurus spp.	propiconazole (f) [8] triadimenol (f) [8]	carbendazim (f) [8]
Jeannenotia stachi (Jeannenot)	carbendazim [8] dimethoate (i) [7] pyrazophos (f) [7]	propiconazole [8] triadimenol [8]
Sminthurides signatus (Krausbauer)	chlorpyrifos (i) [10]	
Sminthurus viridis (L.)	chlorpyrifos [10] dimethoate [7, 10] propiconazole [8] pyrazophos [7] triadimenol [8] intensive regime, wheat [15]	carbendazim [8]
Sminthurinus elegans (Fitch)	carbendazim [8] chlorpyrifos [2] dimethoate [7] propiconazole [8] pyrazophos [7]	triadimenol [8]
Sminthurinus aureus (Lubbock)	dimethoate [7] pyrazophos [7]	methiocarb (m) [12]
Total Sminthuridae	aldicarb (i) [11] gamma-HCH (i)* [11]	
Entomobrya multifasciata (Tullb.)	chlorpyrifos [10] dimethoate [10]	deltamethrin (i) [10]
Lepidocyrtus spp.	carbendazim [8] propiconazole [8] triadimenol aldicarb [11] chlorpyrifos [10] dimethoate [10] gamma-HCH* [11]	cymoxanil + dithianon (f)* [4] deltamethrin [10] dimethoate [7] pyrazophos [7] intensive regime, hops [6]
Pseudosinella alba (Packard)	lambda-cyhalothrin (i) [3] propiconazole [8] triadimenol [8]	carbendazim [8] deltamethrin [10] dimethoate [7, 10] methiocarb [12], pyrazophos [7]
Isotoma viridis Bourlet	carbendazim [8] chlorpyrifos [10] propiconazole [8] triadimenol [8]	dimethoate [7] methiocarb [12] pyrazophos [7]
Isotoma notabilis (Schaffer)	cymoxanil + dithianon* [4] methomyl (i)* [5]	dimethoate [7] lambda-cyhalothrin [3] pyrazophos [7]
Isotomurus palustris (Müller)	chlorpyrifos [10]	cymoxanil + dithianon* [4] dimethoate [7] lambda-cyhalothrin [3] methomyl* [5] methiocarb [12], pyrazophos [7]
Total Isotomidae	gamma-HCH* [11]	
Total Collembola	chlorpyrifos [9, 10] dimethoate [7, 10] oxydemeton-methyl (i) [1] pirimicarb (i) [1] pyrazophos [7, 14]	methiocarb [12, 13]

Monitoring a single easily-recognised species such as *Sminthurus viridis* (e.g. in Vickerman, 1992) would be particularly advantageous to minimise the required taxonomic expertise and sample processing time.

Table 2. *Sources of information referred to in Table 1*

1. Büchs (1991)
2. Çilgi *et al.* (1993)
3. Filser (1992)
4. Filser (1993a)
5. Filser & Nagel (1993)
6. Filser *et al.* (1994)
7. Frampton (1988)
8. Frampton (1989)
9. Frampton & Çilgi (1992)
10. Frampton & Çilgi (1993)
11. Gregoire-Wibo (1983)
12. Kelly & Curry (1985)
13. Martin (1993)
14. Sotherton *et al.* (1987)
15. Vickerman (1992)

Recent work suggests that there may be problems with using individual species because of interspecific variation in effects of pesticides (e.g. Frampton, 1988; 1989; Filser & Nagel, 1993; Frampton & Çilgi, 1993). For instance, effects of organophosphorus pesticides would not have been detected if species only from the sub-order Arthropleona had been monitored by Frampton (1988). At the opposite extreme, recording the total Collembola (all species grouped together) could hide effects on individual species, especially if the most abundant species are less strongly affected than are rarer species, as appeared to be the case in a study of pesticide use in an arable rotation (Heimann-Detlefsen, 1991). A compromise would be to monitor a limited number of species which have been chosen as indicators. Monitoring several species has the advantage that major effects at the community level (Filser *et al.*, 1994) should be detectable. Reference to recent work suggests that a suitable group of species for monitoring would be: *S. viridis*, *Sminthurinus elegans*, *Lepidocyrtus* spp. (principally *cyaneus*) and *Isotoma viridis*. These are among the most abundant and widespread species of epigeal Collembola in UK arable farmland (Frampton, 1989; Frampton & Çilgi, 1993) and reference to Table 1 shows that they are susceptible to a wide range of pesticides in the field. This list could be improved by adding *I. palustris* and *E. multifasciata* (Frampton & Çilgi, 1993), or *I. notabilis* and *P. alba* (Table 1) but there is too little information available at present to judge whether this would be worthwhile, given that these species may occur less widely (G.K.Frampton, unpublished data). These suggestions are summarised in Table 3.

Sampling methods

Sampling methods for epigeal Collembola include pitfall traps, suction samples, sweep nets and soil cores (e.g. Berbiers *et al.*, 1989). Emergence traps have also been used (e.g. Büchs, 1991; 1993) but the results may be difficult to relate to actual densities and species composition in the field. All the species recommended here as potential indicators of pesticide effects may be sampled with a "D-vac" suction sampler (Frampton, 1988; Frampton & Çilgi, 1993) and most can be sampled in pitfall traps (Berbiers *et al.*, 1989; Frampton, 1989). However, sweeping traps predominantly plant-climbing species (such as *S. viridis*) which form a small proportion of epigeal species in cereals (Frampton, 1989) and, although soil cores should provide the most complete picture of the overall community (Berbiers *et al.*, 1989), they may be inefficient at trapping epigeal species. For instance, for most epigeal species or groups in wheat less than one individual per soil core was recovered (Frampton *et al.*, 1992).

The most appropriate methods for sampling epigeal species were concluded to be a combination of suction samples and pitfall traps (Berbiers *et al.*, 1989; Frampton, 1989); the former authors showed that small traps would be needed to assess species composition in the field. Suction sampling is the preferred method as it provides an estimate of density. In winter barley the widely-used "D-vac" suction sampler extracted ≥ 97% of the epigeal Collembola present on the plants and soil surface and in the top soil layer. Also in that study, a higher number of statistically significant effects of fungicides were detected with suction samples than with 7-day pitfall trap samples in a winter wheat crop; effects of fungicides on *S. aureus* were detected only with suction samples (Frampton, 1989). The advantage of using pitfall traps as well as suction samples is that sub-lethal effects on Collembola activity can be detected and other arthropods such as large carabids can also be monitored using the traps.

Table 3. *Suggested species of epigeal Collembola for monitoring to detect adverse effects of pesticides in farmland experiments.*

Level of monitoring	Suggested species
Basic	*Sminthurus viridis, Sminthurinus elegans, Lepidocyrtus cyaneus* or *Lepidocyrtus* spp., *Isotoma viridis*
Detailed	*S. viridis, S. elegans, L. cyaneus* or *L.* spp., *I. viridis, Isotoma notabilis, Isotomurus palustris, Entomobrya multifasciata, Pseudosinella alba*
Full	Individual monitoring of all species present

Number of samples

With data obtained from three years' sampling in cereals in southern England, Frampton (1989) calculated the number of samples that would be required to obtain Collembola population estimates with a known standard error (≤ 20% of the mean) using a power-law relationship between the sample variance and mean (Finch *et al.*, 1978). This method has also been used to draw up sampling plans for Carabidae and Araneae (Vickerman, 1985). The results (Table 4) indicate that an impractically large number of samples would be needed for accurate estimation of Collembola populations except at times of peak abundance and/or activity in the field. At the highest population densities observed, a minimum of three "D-vac" suction samples or five pitfall traps (white plastic cups, 7cm in diameter, 7-day trapping) would have been required to obtain population or activity-density estimates for the suggested indicator species with standard errors ≤ 20% of sample means; at lower Collembola catches over 200 samples would have been required for the same degree of sampling precision (Table 4). These results cannot be extrapolated to all types of pitfall trap (e.g. Berbiers *et al.*, 1989), suction sampler, cropping situation or time of year, but they clearly highlight the problem that in a typical field study using, say, five suction samples (e.g. Frampton, 1988) and ten pitfall traps per experimental treatment (e.g. Frampton, 1989), effects of pesticides will only be detected statistically when species have high abundance and/or activity. Long-term studies may allow effects of pesticides on relatively rare species to be detected by monitoring the persistence of treatment differences over time but short-term studies rely on statistical validation of effects and may fail to detect effects of pesticide treatments on rarer species. Frampton (1988) trapped most Arthropleona in lower numbers than Symphypleona and this could in part explain why effects of dimethoate and pyrazophos were not detected on species of the former sub-order; inadequate sampling intensity is one of the variables that could explain inconsistent results of different studies (Table 1).

Table 4. *Number of samples required for accurate estimation of field Collembola populations (standard error ≤ 20% of the mean) based on data obtained using five suction samples and 10 pitfall traps in winter cereals in summer 1985 to 1987 (from Frampton, 1989; details in text)*

Species	"D-vac" suction samples		Pitfall traps	
	Range of means observed	Number of samples required	Range of means observed	Number of samples required
Sminthurus viridis	0 - 81	3 - 151	0.1 - 11	4 - 164
Sminthurinus elegans	0 - 320	3 - 153	0.1 - 36	5 - 160
Lepidocyrtus cyaneus	0.2 - 372	3 - 177	1.6 - 22	4 - 16
Pseudosinella alba	0 - 95	3 - 207	0.1 - 4	5 - 240
Isotoma viridis	0.1 - 100	3 - 115	0.1 - 7	4 - 233

Sampling periodicity

The frequency with which samples need to be taken will depend upon the type of information required; short-term studies of pesticide side-effects may warrant intensive sampling over weeks or months whereas longer-term studies can provide useful information with one sampling occasion per month (e.g. Frampton & Çilgi, 1993) or per year (e.g. Aebischer, 1991). Ideally, samples should be taken as frequently as possible but this option may be compromised by the resources required for spatial replication of samples (see above) or by other experimental constraints such as habitat damage or the risk of population depletions. Massoud *et al.* (1983) sampled Collembola in woodland and concluded that weekly pitfall trapping sessions were not necessary to obtain reasonably accurate estimates of population changes, but that population changes were not predicted well using sampling sessions more than three weeks apart. In pesticide studies it may be more important to assess the duration of effects on populations than to examine fine-scale temporal population changes. However, it is important to know the limitations of the sampling regime used. A potential limitation of diurnal sampling is that nocturnal species could be under-represented. Pitfall-trapping can overcome this problem by sampling continuously, but changes in the species composition of catches over a 24-hour period would be missed by continuous sampling over several days.

An investigation of diel periodicity of epigeal Collembola in winter wheat (Frampton, 1989) showed that: (1) the relative efficiencies of pitfall trapping, suction sampling and sweeping varied through a 24-h period and from one week to another; (2) for all three sampling methods the species composition of samples changed through a 24-h period and from week to week; (3) most species usually had a diurnal peak in numbers and/or activity, but the time of day when peak catches were made varied between sampling weeks. These results indicate that diurnal catches of Collembola should be interpreted cautiously, particularly if species composition is under investigation. In studies of pesticide effects, however, diel changes in Collembola abundance and/or activity may not be a problem if all treatments are sampled simultaneously on each sampling date.

Experimental designs

Different approaches have been used to investigate side-effects of pesticides on field populations of Collembola and other arthropods in farmland. These can broadly be divided into short-term studies (in one season, usually as replicated plot trials) and long-term studies (over several years). Most field studies have been designed to monitor other arthropods besides Collembola. For instance, in the Boxworth project vertebrate wildlife and plants were also studied (Greig-Smith *et al.*, 1992). Some relevant advantages and disadvantages of these approaches are indicated in Table 5. Long-term studies can show effects of overall pesticide regimes, but it may not be possible to determine the relative importance of the individual constituent pesticides used; in contrast, short-term replicated plot trials may clearly identify effects of individual pesticides but cannot determine the longer-term significance of these. Therefore, these approaches should be used to complement one another. In long-term studies there is a risk that major effects of a few organophosphorus pesticide applications could overshadow more subtle but potentially important direct or indirect effects of other pesticides. For instance, Frampton (1989) detected adverse effects of the fungicides carbendazim, propiconazole and triadimenol in laboratory and replicated-plot field experiments, but effects of these individual fungicides on Collembola have not yet been detected using a different experimental field approach with long-term monitoring of unreplicated experimental areas (Frampton & Çilgi, 1993). As indicated above, there is a need to investigate effects of synthetic pyrethroids and other pesticides such as pirimicarb on Collembola. Long-term studies have not yet provided conclusive evidence for effects of these pesticides on springtails (e.g.Vickerman, 1992; Frampton & Çilgi, 1993) and work involving whole farming systems may confound effects of pesticides with effects of other factors such as tillage or cropping (e.g. Büchs, 1991). Replicated plot experiments would allow effects of synthetic pyrethroids or pirimicarb, but not the long-term consequences of such effects, to be detected.

Table 5. Comparison of short-term and long-term approaches to the study of pesticide effects on Collembola and other arthropods.

	Short-term approach (usually with replicated plots)	Long-term approach
Examples	Gregoire-Wibo (1983) Sotherton *et al.* (1987) Frampton (1988)	Frampton & Çilgi (1992) Greig-Smith *et al.* (1992) Büchs (1993) Filser *et al.* (1994)
Comparison with untreated control	possible	not possible (crop unlikely to be viable in long-term without management)
Detection of effects of individual pesticides	possible	may be possible only for chemicals with major effects
Detection of temporal extent of effects	not possible (duration of studies too short)	possible
Determination of wider ecological consequences	not possible (effects of repeated pesticide use not investigated)	possible
Agricultural realism	usually low (sub-field sized plots often used, with increased risk of recolonisation)	possibly high (may involve whole fields and many pesticides)

Collembola are considered to be poor dispersers in comparison to some other arthropods such as Carabidae but no published rates of dispersal exist for these insects in farmland. Even if they are relatively immobile and unlikely to disperse between experimental treatments, the potential redistribution of their predators (e.g. Carabidae and Linyphiidae) cannot be ruled out. This risk could be minimised by enclosing plots within barriers but Frampton (1989) noted a possible influence of plot barriers on the relative efficiencies of suction sampling and pitfall trapping for *J. stachi* and *S. aureus* and observed that fewer statistically significant effects of fungicides occurred in experimental plots surrounded with barriers than in unenclosed plots.

DISCUSSION

It is not possible to provide sampling plans for all experimental situations. At present, collembolan species may be used to detect effects of actual pesticide use, but cannot be used as models to extrapolate effects to other species or the community. This is because a number of criteria must be satisfied when selecting "model" indicator species (Çilgi, 1994, this volume) and too little is known about the intrinsic susceptibility of different species to pesticides, and their phenology, distribution and ecology in farmland, to select typical species. For example, *Sminthurus viridis* was a good indicator of pesticide use (Vickerman, 1992) but, as one of relatively few species that climb cereal plants (Frampton, 1989), might have been directly exposed to pesticide sprays more often than non-climbing species.

The choice of species as indicators of pesticide use will depend upon the aim of the work (considered here to be the detection of any pesticide effects). A minimum of four species has been proposed as indicators as no single species is affected by all chemicals under all conditions (see Table 1) and because pesticides may affect community composition (Filser *et al.*, 1994), which would require monitoring several species to detect. However, if the purpose of a study is to characterise effects of pesticides in general, it would be necessary to monitor all species (including edaphic species which are not efficiently sampled by the methods recommended above). Detailed monitoring of all species, or all epigeal species (Table 3), would increase the likelihood of detecting pesticide effects but may be too labour intensive. The methods reviewed here may be used more generally to measure epigeal Collembola populations and activity, but the potential limitations of the sampling methods, such as changes in species composition of catches through time, or difficulties in estimating species composition in the field (Berbiers *et al.*, 1989) must be considered when interpreting the results of monitoring.

Information from different investigations would be easier to combine and interpret if studies were standardised where possible, for example in their sampling methods, the species monitored and the reporting of non-significant effects. Some long-term studies may approach agricultural realism but the effects of pesticides may be confounded with other husbandry factors; in contrast, small-plot trials are agriculturally unrealistic (Table 5), but may provide the only means of assessing side-effects of individual chemicals (e.g. for pesticide registration purposes). At present, Collembola are not monitored in pesticide registration work (M.A.F.F. Pesticide Safety Directorate, unpublished) but ongoing long-term studies such as the M.A.F.F. "SCARAB" Project (Frampton & Çilgi, 1993) could provide ecological information which may contribute towards the selection of species of Collembola as predictive bioindicators in future.

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