EFFECTS OF THE FOLIAR FUNGICIDE PYRAZOPHOS ON CEREAL COLLEMBOLA

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#### ABSTRACT

The organophosphorus systemic foliar fungicide pyrazophos significantly increased the mortality of *Sminthurinus aureus* (Collembola: Sminthuridae) in the laboratory. Pyrazophos reduced the numbers of four out of eleven species of Collembola in a field of winter barley and for these species the magnitude of the insecticidal activity of pyrazophos was comparable with that of the broad-spectrum insecticide dimethoate. In both cases, however, significant effects could not be detected in individual species after eleven weeks.

### INTRODUCTION

The use of foliar fungicides has increased steadily since their introduction in the early 1970s. Over 10 million 'spray hectares' (i.e. including more than one spray/field/season) are now treated with foliar fungicides (Hardy, 1986). Some information exists about the side-effects of fungicides on cereal arthropods (Catling, 1969; Reyes & Stevenson, 1975; Vickerman, 1977; Vickerman & Sotherton, 1983; Sotherton & Moreby, 1984). The insecticidal activity of pyrazophos has been demonstrated by various workers but only Sotherton, Moreby & Langley (1987) demonstrated its toxicity to Collembola (as a group), in this case in winter barley.

Since many Collembola are mycetophagous (often facultatively) (Macnamara, 1924; Hale, 1967; Butcher, Snider & Snider, 1971), the collembolan diet is a route for the uptake of systemic fungicides into the cereal microfauna.

Although the importance of Collembola as decomposers is uncertain (Hale, 1967; Butcher, Snider & Snider, 1971) they are numerically important and fall prey to other beneficial invertebrates, particularly the Acarina (Wallace, 1953; Harris, 1975), Araneae (Sunderland, 1986) and Coleoptera (Ernsting & Jansen, 1978; Griffiths, 1983). Where these predators reduce the numbers of insect pests (cereal aphids, for example) there is the risk that the knock-on effects of fungicides on Collembola could influence the balance of pest species in cereals. The aim of this work was to determine whether any of the foliar fungicides commonly used on cereals are toxic to Collembola under agronomically realistic conditions.

## MATERIALS & METHODS

## Laboratory toxicology

Details of the laboratory toxicology are given by Frampton (1988).

The test species, *Sminthurinus aureus*, was taken from a culture which had een maintained under controlled conditions for c. 9 months prior to the toxicological experiments. Substrates, each in an experimental enclosure, were treated with the broad-spectrum fungicide pyrazophos ('Missile', Hoechst U.K. Ltd., 30% a.i.) at a dilution and a rate / unit area equivalent to that recommended for use in the field (2.0 1/ha in 220 1/ha water) using the Potter Precision Spray Tower (Busvine 1971). Control substrates were sprayed with distilled water at a rate/unit area equivalent to 220 1/ha.

Ten adult *S. aureus* were trapped on the treated or control substrate in each experimental enclosure and five substrate replicates were used per treatment. All experimental enclosures were then maintained under identical controlled conditions and the subsequent mortality of the animals was recorded.

Survival curves of control and pyrazophos-treated animals were compared using grouped data proportional hazards models similar to that described by Bartlett (1978). Because of the marked effects of pyrazophos in the laboratory (see Results), this compound was further evaluated in the field.

## Field evaluation

Trial design and fungicide application

The study was conducted in 1985 and the study site is described by Frampton (1988). A 29.5ha field of winter barley (cv Halcyon) was divided into nine plots, each of c. 2.7ha in area. The plots were arranged in a 3 x 3 Latin square design to give three replicates of each of pyrazophos, dimethoate ('Rogor E', Schering Agrochemicals) and control (unsprayed) treatments, dimethoate being used as a potential insecticide toxic standard. Pyrazophos and dimethoate were applied to the appropriate plots on 2 May, 1985 (G.S. 32; Zadoks, Chang & Konzak, 1974) at their recommended field dilutions (pyrazophos 600 g.a.i./ha, dimethoate 400 g.a.i./ha) and rates/unit area (200 1/ha) (Frampton, 1988).

Sampling, extraction and identification of Collembola

A dietrick Vacuum Insect Net (D-vac) was used for sampling epigeal, hemiedaphic and epedaphic Collembola. Ten samples were taken along transects through the centre of each plot and each sample was transferred to 70% alcohol in the laboratory within 24h, after storage at 4°C. Eleven species of Collembola were caught (four symphypleone and seven arthropleone) on five sampling dates: 30 April (pre-treatment), 10 May, 29 May, 13 June and 23 July, 1985. Details of the species caught and methods of their extraction are given by Frampton (1988).

Statistical analysis

A  $\log_{10}$  (<u>x</u>+1) transformation was found to be suitable for the data counts (<u>x</u>). Pre-treatment variability in the numbers of Collembola between experimental plots was taken into account during the subsequent analysis of variance by subtracting the  $\log_{10}$ -transformed mean pre-treatment counts

from their corresponding  $\log_{10}$ -transformed mean post-treatment counts for each species on each date.

#### RESULTS

## Laboratory toxicology

Survival curves for S. aureus after pyrazophos and control treatments are shown in Fig. 1. The curves are significantly different (P<0.001). The effects of pyrazophos were potent with 100% mortality in pyrazophos-treated S. aureus after 13h whereas control animals survived for more than 300h (Fig.1).

### Field evaluation

Of the 11 Collembola species caught, the Latin square analysis of variance revealed significant treatment effects on the four symphypleone species Sminthurus viridis, Sminthurinus elegans, S. aureus and Jeannenotia stachi. The seven arthropleone species were not significantly affected by the pyrazophos treatment (Frampton, 1988) but many of these species were caught in low densities in the control plots.

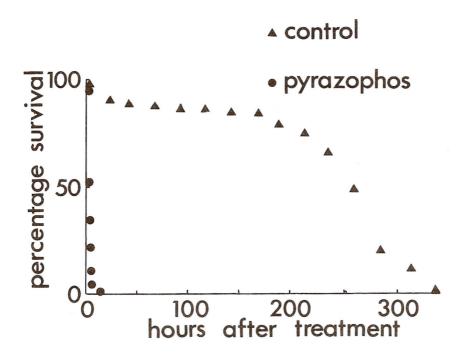


Fig.1. The time-survival relationship for S. aureus following laboratory treatment of the substrate with pyrazophos.

The change in the numbers of the symphypleone species (grouped as the total Symphypleona) relative to the pre-treatment numbers are shown in Fig.2. In the control plots these species reached peak numbers on 13 June. The effects of dimethoate were more immediate than those of pyrazophos; one week after treatment the lowest numbers of these species were found in dimethoate-treated plots whereas six weeks after treatment they were lowest in the pyrazophos-treated plots. The insecticidal activity of pyrazophos on the symphypleone Collembola was similar to that of dimethoate (Fig.2). S. viridis, S. elegans and J. stachi were eliminated from dimethoate and pyrazophos-treated plots on some of the sampling dates (Frampton, 1988) but only in the Symphypleona as a group were significant effects still detected 11 weeks after treatment.

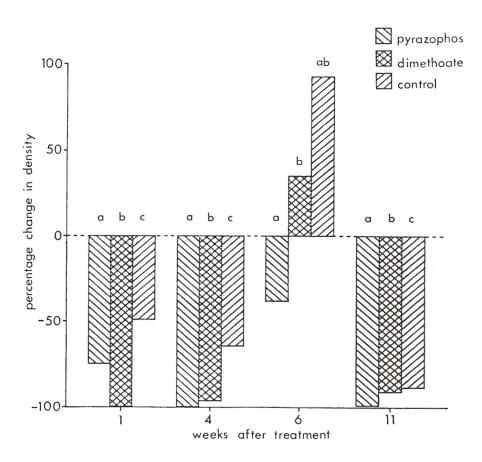


Fig.2. Percentage changes in mean Collembola numbers (per  $\rm m^2$ ) on four post-treatment dates. Positive changes shown an increase in numbers with respect to pre-treatment numbers; negative changes indicate a decrease. All error mean squares calculated from the analysis of variance on  $\rm Log_{10}$  counts were less than 20% of the  $\rm log_{10}$  mean. Histograms sharing the same letter are not significantly different (P<0.05). The densities of control animals on the four dates were 864, 605, 3264 and 198 respectively.

Of the four commonly-used foliar fungicides selected for laboratory screening against *S. aureus*, all were found to increase significantly the mortality of this collembolan. Since the Collembola could have browsed on the treated filter paper substrate or on microorganisms on the treated substrate, it was not possible to determine whether the effects of the fungicides were direct or indirect (via the diet). In the field, the more immediate effects of dimethoate are to be expected since this is a broad-spectrum insecticide (Vickerman & Sunderland, 1977). The lag phase with pyrazophos might be attributed to indirect effects through a reduction in food, for example a reduction in saprophytic fungi.

# Relationship between laboratory toxicology and field evaluation

In the laboratory, substrates were treated with pyrazophos at rates / unit area and dilutions equivalent to those normally used in the field. Under field conditions it is likely that epigeal Collembola will receive a reduced dose of pyrazophos through foliar interception so fungicides not affecting Collembola significantly in the laboratory were considered unlikely to exhibit effects in the field; those exhibiting significant effects on Collembola in the laboratory were considered worthy of further evaluation in the field.

## The field trial design

This study was intended to investigate the action of pyrazophos on Collembola under agronomically realistic conditions. Ideally, the minimum plot size should be an entire field so temporal and spatial redistribution of Collembola within a field would be unlikely to obscure treatment effects. Such large scale experiments are usually prohibitive (Wratten et al.,1988) and in this case the available land was restricted to a 29.5ha field. However, since Collembola are thought to be relatively immobile in comparison with some predatory insects, a large number of small plots would have been acceptable to provide better information on and control of spatial variability in the numbers of Collembola. For small plots, redistribution of Collembola between plots would require monitoring or checking with inclusion-exclusion barriers.

In order to accomodate an independent study of the effects of pyrazophos on mobile predatory insects (Wratten  $et\ al.,1988$ ) at the 29.5ha site, a plot size of c. 2.7ha with 9 plots arranged in a 3 X 3 Latin square matrix was chosen for use by both studies. With the 'large' plot size, the use of inclusion-exclusion barriers was considered unnecessary for the Collembola study and sampling from the centre of each plot was to minimise edge-effects.

## Importance of trends in the results

Eleven weeks after the treatment, no significant effects of pyrazophos could be detected in individual species nor in the total (symphypleone plus arthropleone) Collembola. However, pyrazophos apparently still influenced the numbers of some Collembola species since significantly fewer of the Symphypleona (as a group) were caught in pyrazophos-treated than in control

plots on this date (Fig. 2). This exemplifies the problem that statistical validation of the field effects is only plausible when sufficient densities of animals are caught and where there is reasonable homogeneity between samples. It is therefore important also to consider trends in the numbers of different species even where effects are non-significant. In the four symphypleone species (S. viridis, S. elegans, S. aureus and J. stachi), for example, the numbers caught were always lower in pyrazophos-treated than in control plots eleven weeks after treatment and yet only when the species data were pooled was this effect significant (Fig. 2).

## Comparison with other studies

The results of this field study are supported by those of Sotherton, Moreby & Langley (1987) in which large (>20ha) plots were used. In both studies the effects of pyrazophos on Collembola were detected within one week of treatment and persisted at least for six weeks.

In view of the toxicity of pyrazophos to the Symphypleona demonstrated in this study and its toxicity to other insects (Sotherton, Moreby & Langley, 1987; Wratten  $et\ al$ , 1988) it is surprising that no significant effects of pyrazophos were detected on the Arthropleona.

Of the seven arthropleone species caught, four of these species were much rarer in samples than any of the symphypleone species. Isotoma notabilis, one of the 'rare' arthropleone species, was nevertheless consistently caught in lower numbers in pyrazophos-treated than in control plots. Likewise, the numbers of I.virdis, a species caught in higher densities but with higher spatial variability (perhaps a consequence of its hydrophilic habit) were lower in pyrazophos-treated than control plots four, six and eleven weeks after treatment. The most abundant of the arthropleone species. Lepidocytrus cyaneus, was always caught in lower numbers in pyrazophos-treated plots than in control or dimethoate-treated plots and only in pyrazophos-treated plots did its numbers fall below the pre-treatment levels.

These similarities in the effects of pyrazophos on the symphypleone and on some arthropleone Collembola could be further investigated by comparison of the effects of pyrazophos on insects from each sub-order in the laboratory. Such a comparison between *S. aureus* and *I. viridis* was attempted in the laboratory but was unsuccessful as a result of high *I. viridis* control mortality (G. K. Frampton, unpublished data).

Statistical analysis alone indicates a short-term persistence of pyrazophos except in the Symphypleona (as a group). As stated by Sotherton, Morley & Langley (1987), such apparent short-term persistence does not imply safety of the compound, but merely reflects the scale of the experiment. Although the plot size used in this study was considered acceptable for the study of Collembola, any effects of the redistribution of insects between plots will depend on the duration of the experiment as well as the plot size. In this context it would have been useful to have compared the effects of pyrazophos eleven weeks after treatment in this study with the effects eleven weeks after treatment in the larger plots of Sotherton, Moreby & Langley (1987).

Whilst it is difficult to assess how low densities, variability in numbers and movement of Collembola influenced the effects of pyrazophos on these insects in this field trial, the observed effects will have been mitigated rather than accentuated by these factors. The results presented here therefore do not necessarily represent the maximum effect of pyrazophos on Collembola in a field of winter barley. In view of the potential importance of Collembola in the cereal ecosytem and the continued increase in the use of foliar fungicides in the U.K., a better understanding of the ecology of Collembola in arable land and that of the ecotoxicology of the fungicides is needed.

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