

Evaluation of the Juvenile Hormone Mimic Pyriproxyfen (S-31183) Against Nuisance Chironomids (Diptera: Chironomidae), with Particular Emphasis on *Polypedilum nubifer* (Skuse)

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ABSTRACT Laboratory bioassays of the juvenile hormone mimic pyriproxyfen against late instar larvae of the nuisance chironomid *Polypedilum nubifer* revealed that 0.01 ppm pyriproxyfen caused a 90% inhibition of emergence of this species. A field trial of pyriproxyfen at 0.01 ppm was conducted using *in situ* enclosures. Pyriproxyfen significantly reduced the emergence of *P. nubifer* and another chironomid, *Kiefferulus intertinclus* (Skuse), for 24 d. Larval abundances of these species were not affected by the pesticide application. Pyriproxyfen may provide a satisfactory alternative pesticide to organophosphate control agents currently in use, particularly in highly eutrophic wetlands.

Introduction

The large scale emergence of adult Chironomidae from eutrophic urban wetlands is becoming an increasing problem worldwide (Ali 1991). While these insects are not vectors of any disease organism (Ali 1991), large swarms can cause considerable annoyance and be a persistent nuisance to residents in areas adjacent to wetlands (Pinder *et al.* 1992). In addition, chironomids have been reported to cause economic loss (Ali 1980) and medical problems associated with human allergic reactions (Cranston *et al.* 1983).

In Western Australia the organophosphate larvicide temephos has been the primary means of chironomid control for over two decades. However, the declining effectiveness of this compound has emphasised the need to investigate alternative control options. Insect growth regulators (IGRs) such as the juvenoid methoprene and the chiton synthesis inhibitor diflubenzuron are currently in use against chironomids in Japan and the latter is registered in California. A more recently developed IGR, the juvenile hormone mimic pyriproxyfen (S-31183; {2-[1-methyl-2-(4-phenoxyphenoxy) ethoxyl] pyridine) is reportedly more effective against mosquitoes than either of these compounds (Estrada and Mulla 1986; Amalraj *et al.* 1988). While the efficacy of pyriproxyfen has been evaluated against a range of dipteran pests including mosquitoes (e.g. Estrada and Mulla 1986), the housefly (Kawada *et al.* 1987) and the tsetse fly (Langley *et al.* 1990), no studies have directly assessed its effectiveness against chironomids.

The objective of this study was to investigate the activity of pyriproxyfen against chironomids, particularly *Polypedilum nubifer* (Skuse), a major nuisance species in Western Australia. Laboratory bioassays were conducted to determine an effective rate of this compound against *P. nubifer* and then that rate was tested under field conditions using *in situ* enclosures.

Methods

Test material. Both laboratory and field trials utilised a granular formulation of pyriproxyfen consisting of 0.5% w/w of the active ingredient on Ishikawarite granules (Sumitomo).

Laboratory assays. The biological activity of pyriproxyfen was tested against late instar *P. nubifer* larvae. Three separate assays were performed and each involved testing five different concentrations of pyriproxyfen ranging from 0.001 to 0.02 ppm and a control solution, with three replicates of each. Tests were carried out at room temperature in aquaria (35 × 25 × 20 cm) containing 2 cm of washed sand and 8 L of water. Both the water and the larvae used in the assays were collected from local wetlands. Fifty late instar *P. nubifer* larvae were added to each test solution and wire mesh (1.5 mm) was placed over the top of the tanks to stop emergent midges escaping. The aquaria were aerated during the day but not at night in order to prevent any disturbance of emergence. The aquaria were monitored daily and dead pupae and live or dead adults were removed. The assays were terminated when pupal mortality in the control aquaria reached 20% (Russell 1986). The activity of pyriproxyfen was assessed as the percentage inhibition of emergence calculated by comparing the total emergence from each test solution with that of the control aquaria. Data were analysed using probit analysis (SPSS 1988).

Field trial. The field trial took place late in the spring of 1989 at North Lake, a eutrophic wetland located approximately 14 km south of Perth (115° 49'E, 32° 4'S). The experiment utilised six enclosures which were located in the littoral region on the eastern side of the lake. Each enclosure surrounded an area of water 25 m² and comprised a clear, polyethylene plastic sheet (200 µm thick) supported at the top and bottom by PVC pipes. The pipes at the bottom were weighted and followed the contours of the lake bed, while the pipes at the top were tied to star pickets 50 cm

above the water surface. The enclosures were erected 7 d prior to pesticide application. Pyriproxyfen was added to three randomly chosen enclosures at the rate of 50 g [A.I.]/ha (equivalent to 0.01 ppm in the enclosed water). The remaining enclosures acted as controls. Emerging chironomids were monitored using submerged funnel traps, similar to those used by

Pinder *et al.* (1993). Emergence was monitored every night for 6 nights prior to the application of the pesticide and then every second night for a 15 d period. Thereafter emergence was monitored every third night until completion of the experiment, 9 d later. On each occasion, three traps were set in each enclosure in the mid-afternoon and collected mid-morning the following day. Traps were never set in the same position on consecutive sampling occasions. Chironomids collected in the traps were preserved with 100% ethanol and later counted and identified to species.

Larval chironomids were monitored by taking three sediment samples from each enclosure using a corer (10 cm diameter). These were collected 6 d and then 1 d prior to the application and 7 and 20 d afterwards. The samples, which were preserved in 70% ethanol, were later sorted, counted and identified to species.

Water temperature and dissolved oxygen concentration were measured using an oxygen probe (YSI model 58) and water samples (600 mL) from each enclosure were collected on each occasion when larval chironomids were sampled. The pH and conductivity of each sample was measured using Hanna meters and 60-70 mL was filtered under vacuum through glass microfibre filters, stored at -20°C and later analysed for chlorophyll-*a* content according to the methods of Moran and Porath (1980).

The mean emergence rate and larval density of each enclosure were used to test the effect of pyriproxyfen using separate repeated measures analyses of variance (ANOVAR) for each chironomid species (SPSS 1988). ANOVAR enables orthogonal contrasts of interest to be examined. In this case, the contrast of most relevance was the difference between the emergence rate from each enclosure type on all of the pre- and post-treatment sampling occasions. A significant interactive effect would be expected to occur if the pesticide had a detrimental effect on chironomid emergence or larval abundance in the treated enclosures.

All dependent variables were tested for heteroscedasticity using Cochran's C-test and those variables showing significant ($P < 0.05$) heterogeneity were log transformed [$\log_{10}(n+1)$]. The results of the analyses were accepted as significant at $P < 0.05$ except where Cochran's C test showed that the variance was still heterogeneous after transformation. Where this occurred the results were only considered to be significant if $P < 0.01$ (Underwood 1981).

Results

Laboratory assays. Probit analysis using data from the laboratory assays indicated that pyriproxyfen achieved a 90% inhibition of emergence (EI90) of *P. nubifer* with a concentration of 0.01 ppm (slope = 1.68).

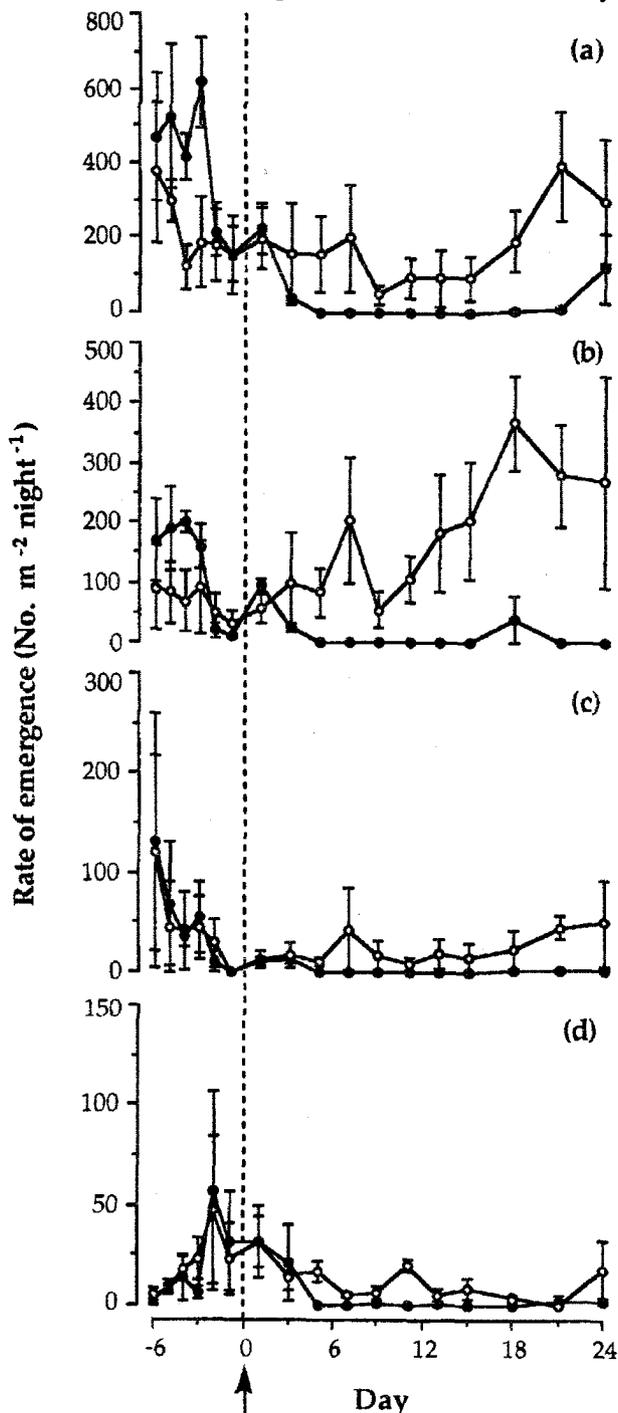


Fig. 1. Mean rate of emergence (\pm s.e.) of adult chironomids from the treated (●) and control (○) enclosures: (a) *Polypedilum nubifer*; (b) *Kiefferulus interinctus*; (c) *Chironomus aff. alternans*; (d) *Cryptochironomus griseidorsum*. The arrow indicates the application of pyriproxyfen.

Field trial. While *P. nubifer* was the most abundant species in the emergence traps, *Kiefferulus intertinctus* (Skuse), *Chironomus* aff. *alternans* (Walker) and *Cryptochironomus griseidorsum* (Kieffer) were also common. The responses of these four species to the pyriproxyfen application were similar, with the rate of emergence from the treated enclosures declining to zero within 5 d of the application and subsequently remaining below that of the control enclosures (Fig. 1). Results of the ANOVAR indicated that the post-treatment reduction in emergence of *P. nubifer* and *K. intertinctus* was significant ($P < 0.01$, $F = 46.73$ and 48.57 respectively; d.f. = 1,3). The emergence of these two species was suppressed for 24 d.

P. nubifer larvae were abundant in the enclosures with densities frequently above 5,000 larvae/m² (Fig. 2). Densities of this species were similar in the treated and control enclosures throughout the experiment with the application of the pesticide having no significant effect ($P > 0.05$) upon larval density in the treated enclosures. Densities of larval *K. intertinctus*, *C. aff. alternans* and *C. griseidorsum* showed greater variation between treated and control enclosures before and after the application of pesticide (Fig. 2) which had no significant detrimental effect on the abundance of these species ($P > 0.05$).

The physico-chemical characteristics of the treated and control enclosures did not differ considerably either before or after the pesticide application. The concentrations of chlorophyll-*a* and dissolved oxygen in both treatment types fluctuated between 100 to 250 µg/L and 7 to 11 mg/L, respectively. While water temperature remained relatively constant (23-25 °C), conductivity ranged from 550 and 650 µS/cm and pH from 8 to 9.5 in both the treated and control enclosures.

Discussion

The laboratory trials indicated that, under controlled conditions, a rate of 0.01 ppm pyriproxyfen would achieve a 90% inhibition of emergence of *P. nubifer*. Under field conditions, an equivalent rate of application suppressed the emergence of *P. nubifer* from the treated enclosures for 24 d. This effect could not be attributed to changes in environmental variables since conditions in the treated enclosures were similar to the controls, throughout the experiment.

Pyriproxyfen also inhibited the emergence of the chironomid *K. intertinctus*. However, low numbers of emergent *C. griseidorsum* and *C. aff. alternans* meant that any effect of the pesticide on these species could not be demonstrated statistically. The application had no effect upon the larval abundance of any of the four species, which conforms with results reported by Schaefer and Muira (1990) using the same rate of application. This effect is consistent with that of many insect growth regulators and can be considered highly

desirable since it has a less dramatic effect upon wetland food chains than the action of organophosphate larvicides (Ali 1991).

The field experiment was terminated 24 d after the pesticide application when chironomid emergence from the treated enclosures was still below that of the controls. As a consequence, it was not possible to determine the period over which emergence would have recovered. Pyriproxyfen is reported to

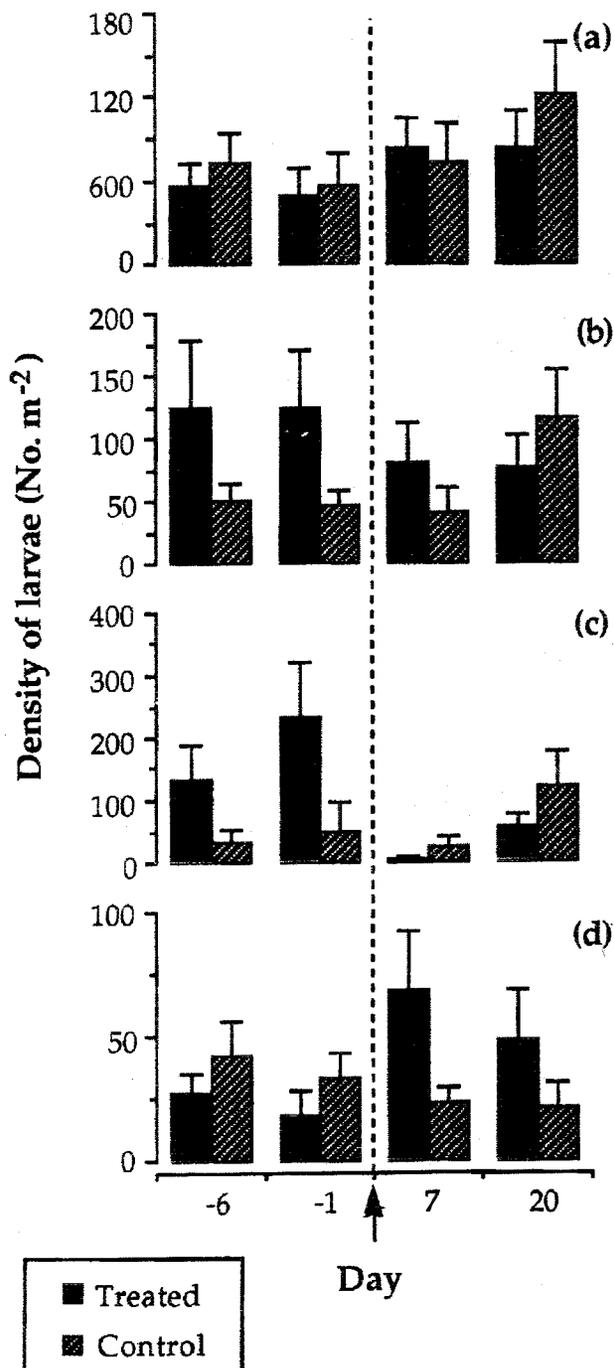


Fig. 2. Mean densities (\pm s.e.) of chironomid larvae in the control and treated enclosures: (a) *Polypedilum nubifer*; (b) *Kiefferulus intertinctus*; (c) *Chironomus* aff. *alternans*; (d) *Cryptochironomus griseidorsum*. The arrow indicates the application of pyriproxyfen.

have long periods of residual activity which is prolonged with increased organic content in the water column and has been known to exceed 2 months in highly polluted water bodies (Schaefer *et al.* 1988; Mulligan and Schaefer 1990). This particular attribute may be of benefit to control programs in areas of poor water quality since the effectiveness of temephos is reduced under such conditions (Miles and Woehst 1969). Thus pyriproxyfen offers good potential as control agent for the nuisance chironomid, *P. nubifer*, as well as other chironomid species, particularly in the highly eutrophic urban wetlands which these dipterans frequent.

Pyriproxyfen has limited bioaccumulative ability (Schaefer *et al.* 1988; Schaefer and Muira 1990) and appears to be of low toxicity to mammals (LD₅₀ rats > 2,000 mg/kg) and fish (96 h LC₅₀ carp 832 mg/L; Sumitomo unpublished data). However, Schaefer and Muira (1990) found that an application of pyriproxyfen to field plots at 50 g [A.I.]/ha (0.01 ppm water concentration) caused a significant reduction in the reproductive capacity of daphnid cladocerans and induced morphometric aberrations in emergent dragonflies. Thus, it is possible that some non-target insects and crustaceans may be detrimentally affected by the rates of pyriproxyfen which controlled *P. nubifer* in this study. The potential for harmful effects of pyriproxyfen upon non-target fauna, therefore, warrants further investigation.

Acknowledgments

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