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Effect of field-weathered residues of pyriproxyfen on the predatory coccinellids *Chilocorus circumdatus* Gyllenhal and *Cryptolaemus montrouzieri* Mulsant

K. M. Smith[A], *D. Smith*[B]*, and A. T. Lisle*[A]

[A]School of Land and Food, University of Queensland, Gatton, Qld 4345, Australia; e-mail: alisle@uqg.uq.edu.au
[B]Queensland Horticultural Institute, Maroochy Research Station, Nambour, Qld 4560, Australia; SmithD2@dpi.qld.gov.au

Summary. The residual toxicity of field-weathered residues of the juvenile hormone analogue insecticide, pyriproxyfen was evaluated against two coccinellids, *Cryptolaemus montrouzieri* Mulsant and *Chilocorus circumdatus* Gyllenhal, key predators of mealybugs and scales in citrus in southeast Queensland.

Pyriproxyfen was applied as a high volume spray at 0.1, 1, 10, 25, 50 and 100 mg/L to late Valencia orange trees, and larvae and adults of *C. montrouzieri* and *C. circumdatus* were exposed to leaves picked at intervals varying from 0 to 112 days after spraying. The effect on fourth and fifth instar larvae and pupation and subsequent adult emergence, and on the viability of eggs from treated adults, was measured. Regression models were developed to relate larval mortality and egg hatch to pyriproxyfen concentration and weathering time.

Pyriproxyfen killed larvae or prevented pupation of 50% of *C. montrouzieri* for 64 days at 10 mg/L and for a predicted 167 days at 100 mg/L; the effect on *C. circumdatus* was even greater, lasting a predicted 210 days at 10 mg/L.

Egg-hatch (from adults exposed to treated leaves) was suppressed at 10 mg/L for 28 days in *C. montrouzieri* and for 50 days in *C. circumdatus*; at 100 mg/L, suppression extended to 50 days and a predicted 478 days, respectively.

The combined effects on larvae and eggs of rates between 10 and 100 mg/L would be extremely disruptive to both species. Disruption was much less at 2 mg/L (lasting up to 3 weeks) and at 5 mg/L (lasting up to 7 weeks). Because of its potency against scale insects, pyriproxyfen may yet have a role in Integrated Pest Management (IPM) providing it is used very sparingly at dosage rates no greater than 2 mg/L.

Additional keywords: Coccinellidae, integrated pest management, scale insects, biological control.

Introduction

Pyriproxyfen, 2–(1-methyl-2 (4-phenoxyphenoxy) ethoxy pyridine (MPEPP) is a juvenile hormone analogue affecting hormonal balance and chitin deposition in juvenile insect stages and causing deformation and death at moulting and pupation. It is used as an insecticide to kill larvae, and treated adults may lay eggs that fail to hatch (Cohen 1987; Kawada *et al.* 1989; Ishaaya 1990; Dhadialla *et al.* 1998).

Pyriproxyfen is strongly insecticidal to diaspid scale insects including red scale, *Aonidiella aurantii* (Maskell) and coccid scales such as Florida wax scale, *Ceroplastes floridensis* Comstock at concentrations of 25–100 mg/L (Peleg 1988). In South Africa it is used commercially to control red scale in citrus at 30 mg/L, in

combination with mineral oil at 0.3% (Hattingh and Tate 1995). It is also effective against sweet potato whitefly, *Bemisia tabaci* (Gennadius) at rates from 0.01–1 mg/L, (Ishaaya and Horowitz 1992) and aphids [(*Schizaphis graminum* (Rondani) (Nasser *et al.* 1973)]. Other insects controlled include cockroaches [(*Blatella germanica* (Linnaeus) (Kawada *et al* 1989)], tsetse fly, *Glossina morsitans* Linnaeus and house fly, *Musca domestica* Linnaeus (Ishaaya and Horowitz 1992; Langley 1990), termites [(*Coptotermes formosanus* Shiraki) (Koehler and Patterson 1991)] and some lepidoptera [(codling moth, *Cydia pomonella* (Linnaeus) (Yokoyama and Miller 1991)]. The residual activity of pyriproxyfen against some of these pests is remarkable — 3.8 mg/m^2 remains active against cockroaches for up to 12 months

(Kawada *et al* 1989), while sheets impregnated with the compound induce sterility in tsetse flies for up to 2 years (Langley 1990).

Integrated pest management (IPM) is widely practiced in citrus in Queensland and other parts of Australia (Smith *et al.* 1997). Because of the importance of scale pests of citrus (particularly red scale), insecticides with low toxicity to scale parasitoids are an attractive supplement to biological control. Insect growth regulators (IGRs) like pyriproxyfen have low toxicity to mammals, fish and birds, in addition giving good scale control, and sufficient specificity to make them useful in IPM (Ishaaya 1990; Dhadialla *et al.* 1998).

There is increasing evidence, however, to suggest they kill some natural enemies. Hattingh and Tate (1995, 1996) and Hattingh (1996) claim that the extensive use of pyriproxyfen for the control of red scale in South Africa has led to the disruption of biological control of cottony cushion scale, *Icerya purchasi* Maskell, due to adverse effects on the coccinellid *Rodolia cardinalis* (Mulsant). Disruption of *R. cardinalis* has also been reported from Mediterranean areas (Loia and Viggiani 1992; Mendel *et al.* 1994). *Chilocorus nigrita* (Fabricius), another important predatory coccinellid in South Africa, has also suffered from the use of pyriproxyfen (Hattingh and Tate 1995). They claim there is evidence of long-term disruption of both ladybirds throughout whole districts in South Africa, and studied the effect of weathered residues on *C. nigrita* and *Cryptolaemus montrouzieri* Mulsant, to acquire data to explain their observations. Pyriproxyfen applied at 30 mg/L prevented egg hatch in *C. nigrita* for 19 weeks and for 1 week in *C. montrouzieri*. They concluded that pyriproxyfen was not compatible with citrus IPM in South Africa, and expressed fears for other beetle species within the general environment (Hattingh 1996). Grafton-Cardwell (1998) found pyriproxyfen to be toxic to larvae of the coccinellid *Rhyzobius lophanthae* (Blaisdell) for at least 12 weeks.

Mendel *et al.* (1994) also report disruption of the scale predators *Cryptochaetum iceryae* Williston (Diptera) and *Elatophilus herbraicus* Pericart (Hemiptera) after exposure to pyriproxyfen.

Most research, however, has so far shown limited disruption by pyriproxyfen (and other IGRs) to hymenoptera (Peleg 1988; Horowitz and Ishaaya 1994; Smith 1995; Smith and Papacek 1995; Liu and Stansly 1997).

The main concern with pyriproxyfen in IPM programs has been its extreme persistence in the field and its consequential adverse effect on coccinellids (Horowitz and Ishaaya 1994; Hattingh 1996). In this study, 2 important coccinellid predators in citrus in southeast Queensland are exposed to pyriproxyfen residues which had weathered for up to 5 months. The effect on fourth and fifth instar larvae, pupation and subsequent adult emergence, and on the viability of eggs from treated adults, was measured.

C. montrouzieri is a key predator of citrus mealybug, *Planococcus citri* (Risso) and soft scales such as cottony citrus scale, *Pulvinaria polygonata* Cockerell (Smith *et al.* 1997), while *Chilocorus circumdatus* Gyllenhal, controls citrus snow scale, *Unaspis citri* (Comstock) (Smith and Papacek 1995).

Materials and methods

In April 1998, 6 random 2-m high, late Valencia orange trees in a 0.5-ha citrus block at Maroochy Research Station, Nambour were each thoroughly sprayed with pyriproxyfen at 0.1, 1, 10, 25, 50 and 100 mg/L (using a 10% emulsifable concentrate) together with narrow range petroleum oil (Caltex Loviz) at 3 mL/L of water. Petroleum oil alone was also applied at the same rate and untreated trees were included as controls. Sprayed trees were widely separated and protected by guard trees to prevent contamination from spray drift.

Insectary cultures of *C. montrouzieri* and *C. circumdatus* were maintained at 25°C on citrus mealybug and oleander scale *Aspidiotus nerii* Bouche, respectively, which were reared on butternut pumpkins. The sex of adult *C. montrouzieri* was determined by the colour of the forelegs — black in females, yellow in males (Hattingh and Tate 1995).

Adult female *C. circumdatus* were selected based on their larger size and differences in the margin of the sixth visible sternite (Hodek 1973).

Adult beetles or larvae were exposed to treated citrus leaves picked at defined times after spraying, from 0 (30 min with the leaves just dry) to 112 days (Tables 3 and 4).

The picked leaves were stored for use in plastic freezer bags and kept at 5°C. Adult beetles were pre-exposed to the treated leaves for 24 h in a 4-L ice-cream tub with a mesh lid before introducing them into the test arenas with their food host.

The test arenas for larval *C. montrouzieri* were plastic tubes 8 cm long by 3 cm in diameter with mesh lids. One large leaf (rolled into a cylindrical shape) was placed in each tube and 5 fourth or fifth instar larvae were introduced and left for 24 h before the introduction of a large cluster of adult mealybugs and eggs. To prevent mould growth in these narrow tubes, each was connected to a central plastic conduit through which air was pumped constantly via an aquarium pump. After 3 days, a fresh cluster of mealybugs and eggs was introduced. The number of healthy adult beetles (maturing from the exposed larvae) in each tube was counted after 3 weeks.

The test arenas for adult *C. montrouzieri*, were small plastic cups, 3 cm high by 4 cm diameter fixed to whole butternut pumpkins, uniformly covered with citrus mealybug. The base of each was removed and replaced with fine mesh for ventilation. The space between the mesh and the surface of the pumpkin was filled

with 1 or 2 of the test leaves before a pair of 1-week-old pre-exposed adults (male and female) was introduced. Test leaves were replaced with fresh leaves from the freezer bags after 3 days and after a further four days the leaves and beetles were removed. The number of larvae (successfully hatching from the deposited eggs) in each cup was counted after another 10 days.

The test arenas for larval *C. circumdatus* were 8 cm high by 8 cm diameter plastic tubs with mesh lids. The tubs were filled with test leaves and 5 fourth or fifth instar larvae were introduced into each tub. After 24 h, a 6-cm long sliver of butternut pumpkin infested with oleander scale was introduced. A fresh piece was added after 3 days and after 3 weeks the number of healthy adult beetles (maturing from the exposed larvae) in each tub was counted.

The test arena for adult *C. circumdatus*, was a 4-L ice-cream tub with a mesh lid. The tub was half-filled with test leaves, followed by a whole 1 kg butternut pumpkin, which was uniformly covered with oleander scale. This was then covered with more leaves and 10 pairs of pre-exposed 1-week-old beetles were introduced. The leaves and the beetles were removed after 7 days and the number of larvae (successfully hatching from the deposited eggs) was counted after a further 10 days.

All of the tests were conducted at 25°C and replicated at least 3 times. Rainfall and temperature data were recorded daily during the trials.

There were no differences in larval survival and hatch rate from the untreated and oil only controls so the data for each species were combined, since preliminary analyses showed no differences between the control treatments. For mathematical convenience, the leaf samples taken immediately after spraying were coded as 0.1 days.

Logistic regression analyses allowing for natural mortality (Collett 1991) were conducted on the proportion of larvae of each species surviving to pupation and adult emergence. Analyses were carried out using the SAS GENMOD procedure. The fitted models were then rearranged to predict the necessary weathering time to achieve 50% larval survival for any given concentration.

The numbers of larvae hatching were first of all scaled by dividing by the average larval hatch from the combined controls. This relative hatch rate was then modelled using a logistic relationship, similar to that used for larval survival, except that models were fitted by non-linear regression using the SAS NLIN procedure. Again, the fitted models were rearranged to predict the necessary weathering time to achieve a relative hatch rate of 50% for any given concentration.

Results

For larval survival (and successful pupation and emergence), the logistic regression model, allowing for natural mortality is:

$$p_i = (1 - \pi_0)/(1 + e^{-\beta X})$$

where p_i is the proportion of larvae surviving and π_0 is the natural mortality. The independent variables βX can be expanded as $\beta_0 + \beta_1 T_i + \beta_2 C_i + \beta_3 C_i T_i$, where C_i is $\log_{10}$(concentration in mg/L) and T_i is $\log_{10}$(weathering time).

For egg-hatch, the logistic model fitted to the relative hatch rates (RHR) was:

$$RHR = 1/(1 + e^{-\beta X})$$

where, once again, βX can be expanded as $\beta_0 + \beta_1 T_i + \beta_2 C_i + \beta_3 C_i T_i$.

The models for larval survival (Table 1) provided an adequate fit to the observed data, and the residual deviance was not significant in either case. The models can be rearranged to express T in terms of C for any survival rate. Choosing 50% induced mortality (excluding natural mortality), gives:

$$T = - (\beta_0 + \beta_2 C)/(\beta_1 + \beta_3 C)$$

The required weathering times were calculated for the 6 trial rates of pyriproxyfen together with rates of 2 and 5 mg/L (Table 2).

In the models for egg-hatch (Table 1), the proportion of between group variation (R^2) explained by the fitted models was 0.84 (*C. montrouzieri*) and 0.91 (*C. circumdatus*).

As with larval survival, we can express T in terms of

Table 1. Larval survival and egg hatch models for *C. montrouzieri* and *C. circumdatus*

Values in parentheses represent standard errors of the estimated parameters

Coccinellid sp.	Baseline values		Independent variables (βX) for logistic regression
	Mortality	Egg-hatch	
			Larval survival
C. montrouzieri	0.209 (± 0.027)		-2.758 (± 0.708) $- 6.639$ (± 1.201)C $+ 3.175$ (± 0.650)T $+ 2.021$ (± 0.622)CT
C. circumdatus	0.200 (± 0.033)		-0.470 (± 0.394) $- 3.138$ (± 0.510)C $+ 1.551$ (± 0.379)T†
			Relative hatch rate
C. montrouzieri		13.19 (± 0.68)	-7.284 (± 2.040) $- 8.327$ (± 2.074)C $+ 5.699$ (± 1.425)T
C.circumdatus		58.53 (± 2.89)	-1.062 (± 0.182) $- 1.462$ (± 0.127)C $+ 1.488$ (± 0.154)T†

†The parameter associated with the CT interaction term (for *C. circumdatus*) was not significantly different from zero and was omitted from the model.

Table 2. Estimated weathering time (days) to obtain 50% larval survival and 50% egg-hatch for *C. montrouzieri* and *C. circumdatus* at different rates of pyriproxyfen

Pyriproxyfen applied (mg/L)	*C. montrouzieri*		*C. circumdatus*	
	Larval survival	Egg-hatch	Larval survival	Egg-hatch
0.1	—	—	—	0.5
1	7.4	19.0	2.0	5.2
2	18.1	22.5	8.2	10.2
5	41.0	25.8	52.2	25.2
10	64.3	27.7	212.3	49.7
25	101.5	29.6	1356.2	122.4
50	133.1	30.7	5514.5	242.0
100	166.7	31.7	22423.3	478.3

C in order to estimate the weathering time necessary to achieve a relative hatch rate of 50% (Table 2).

The mean percentage larval survival and numbers of eggs hatching for *C. montrouzieri* and *C. circumdatus* with standard errors are shown in Tables 3 and 4.

Rainfall during the 6-month period of the trials (April–September 1998) totaled 717.8 mm and maximum – minimum temperature averages for April were 26.2 and 16.8°C and for July 21.6 and 10.7°C.

Discussion

Hattingh and Tate (1995) used a modified Mungar cell to ensure maximum exposure of the beetles to the test leaves. In this study, the necessity to supply live mealybugs and scales growing on a living host made the use of very small arenas impractical because of heavy fungal growth on the pumpkin. A range of arenas were trialled and those adopted were considered to give the most consistent results.

The effect of some IGRs on moulting of juvenile stages and pupation is well documented (Ishaaya 1990). Pyriproxyfen residues were very persistent and toxic to larvae of both beetles in this study. Interference with larval moulting occurred but effects on pupation and adult emergence were more noticeable. A single application of pyriproxyfen at 100 mg/L is reported to

Table 3. Mean (± s.e.) larval survival (as a proportion) and relative egg-hatch rates for *C. montrouzieri* exposed to leaves treated with different rates of pyriproxyfen and weathered for up to 112 days

No. of days	Pyriproxyfen applied (mg/L)					
	0.1	1.0	10	25	50	100
	Proportion of larvae surviving					
0.1	0.750 ± 0.097	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
4	0.750 ± 0.097	0.250 ± 0.097	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
14	0.600 ± 0.126	0.533 ± 0.129	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
28	0.933 ± 0.064	0.733 ± 0.114	0.067 ± 0.064	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
42	1.000 ± 0.000	0.667 ± 0.122	0.267 ± 0.114	0.133 ± 0.088	0.000 ± 0.000	0.000 ± 0.000
56	0.933 ± 0.064	0.733 ± 0.114	0.333 ± 0.122	0.200 ± 0.103	0.000 ± 0.000	0.000 ± 0.000
112	0.867 ± 0.088	0.933 ± 0.064	0.533 ± 0.129	0.400 ± 0.126	0.000 ± 0.000	0.000 ± 0.000
	Relative egg hatch rate					
0.1	0.708 ± 0.178	0.278 ± 0.178	0.202 ± 0.178	0.076 ± 0.178	0.000 ± 0.178	0.000 ± 0.178
4	0.607 ± 0.178	0.303 ± 0.178	0.303 ± 0.178	0.101 ± 0.178	0.101 ± 0.178	0.000 ± 0.178
14	0.708 ± 0.178	0.404 ± 0.178	0.152 ± 0.178	0.152 ± 0.178	0.126 ± 0.178	0.177 ± 0.178
28	0.758 ± 0.126	0.569 ± 0.126	0.404 ± 0.126	0.594 ± 0.126	0.253 ± 0.126	0.291 ± 0.126
42	0.809 ± 0.178	0.859 ± 0.178	1.163 ± 0.178	1.011 ± 0.178	0.859 ± 0.178	0.758 ± 0.178
56	1.365 ± 0.178	1.213 ± 0.178	1.011 ± 0.178	1.011 ± 0.178	0.961 ± 0.178	0.961 ± 0.178
112	1.087 ± 0.178	1.062 ± 0.178	1.036 ± 0.178	1.011 ± 0.178	0.935 ± 0.178	0.885 ± 0.178

Table 4. Mean (± s.e.) larval survival (as a proportion) and relative egg-hatch rate for *C. circumdatus* exposed to leaves treated with different rates of pyriproxyfen, weathered for up to 84 days

No. of days	Pyriproxyfen applied (mg/L)					
	0.1	1.0	10	25	50	100
Proportion of larvae surviving						
0.1	0.600 ± 0.126	0.200 ± 0.103	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
14	0.800 ± 0.103	0.267 ± 0.114	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
28	0.733 ± 0.114	0.667 ± 0.122	0.267 ± 0.114	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
56	0.933 ± 0.064	0.733 ± 0.114	0.267 ± 0.114	0.133 ± 0.088	0.000 ± 0.000	0.000 ± 0.000
84	0.733 ± 0.114	0.667 ± 0.122	0.600 ± 0.126	0.133 ± 0.088	0.000 ± 0.000	0.000 ± 0.000
Relative egg-hatch rate						
0.1	0.234 ± 0.056	0.152 ± 0.056	0.019 ± 0.056	0.031 ± 0.056	0.010 ± 0.056	0.006 ± 0.056
14	0.741 ± 0.056	0.754 ± 0.056	0.396 ± 0.056	0.076 ± 0.056	0.105 ± 0.056	0.011 ± 0.056
28	0.926 ± 0.056	0.648 ± 0.056	0.488 ± 0.056	0.195 ± 0.056	0.154 ± 0.056	0.152 ± 0.056
56	0.740 ± 0.080	0.938 ± 0.080	0.524 ± 0.080	0.563 ± 0.080	0.409 ± 0.080	0.177 ± 0.080
84	0.771 ± 0.080	0.966 ± 0.080	0.545 ± 0.080	0.591 ± 0.080	0.149 ± 0.080	0.129 ± 0.080

give effective control of red scale for two seasons in Israel (Hattingh pers. comm.). The predicted time to achieve 50% survival for the 100 mg/L rate in these trials was 167 days for *C. montrouzieri* and was unable to be accurately predicted for *C. circumdatus*. (Pyriproxyfen was considerably more toxic to *C. circumdatus* than *C. montrouzieri*.) It is apparent from Table 2 that rates above 2 mg/L would be very disruptive to the larvae of these predators.

There was also a persistent effect on egg-hatch, again particularly of *C. circumdatus* at rates of 10 mg/L and higher. Hattingh and Tate (1995) found that pyriproxyfen at 30 mg/L affected egg-hatch in *C. montrouzieri* for 7 days (in tests of weathering ages of 7 and 70 days) and *C. nigrita* for 131 days. They found that egg production was not significantly affected and in this study, this parameter was not investigated. Hattingh and Tate (1995) also observed that normal egg-hatch resumed 20 days after adults were no longer exposed to separate residues. IGRs like pyriproxyfen act on juvenile stages and usually have low toxicity to adult stages (Ishaaya 1990) and no significant mortality of adult beetles occurred during this study.

The combined persistent effects of pyriproxyfen against larval survival and egg-hatch of *C.montrouzieri* and *C. circumdatus*, however, indicates that its use at rates above 2 mg/L in citrus in Queensland would be extremely disruptive to an IPM program. There would be a strong possibility of outbreaks of citrus mealybug, cottony citrus scale, cottony cushion scale and citrus snow scale. A wide range of other scale, aphid, whitefly and mite feeding coccinellids could also suffer (Smith *et al.* 1997) and multiple applications would compound the effect.

Preliminary studies with pyriproxyfen used against red scale show that rates as low as 1 mg/L are effective against juvenile stages (D. Smith unpublished data). Grafton-Cardwell (pers. comm.) reports that it is used at similar low rates for red scale in California. If used sparingly at these low rates against red scale, it could be a useful supplement to biological control.

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