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Effects of field-weathered residues of insect growth regulators on some Coccinellidae (Coleoptera) of economic importance as biocontrol agents

Vaughan Hattingh and Bruce Tate

Outspan Citrus Centre, Nelspruit, South Africa

Abstract

Use of the insect growth regulator (IGR) pyriproxyfen (Nemesis®) for the control of red scale *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae) on citrus in southern Africa has led to extensive disruption of the biocontrol of cottony cushion scale *Icerya purchasi* Maskell (Homoptera: Margarodidae) provided by the coccinellids *Rodolia cardinalis* (Mulsant) and other indigenous *Rodolia* spp. Similar effects on field populations of *Chilocorus nigrita* (Fabricius), a coccinellid predator of *A. aurantii*, have also been observed. The adverse effects of field-weathered residues of IGRs on the fecundity and egg viability of the coccinellids *C. nigrita* and *Cryptolaemus montrouzieri* Mulsant were determined in a laboratory bioassay. Residues of pyriproxyfen, a juvenile hormone analogue, and two chitin synthesis inhibitors, buprofezin (Applaud®) and triflumuron (Alsystin®), were tested. Exposure to residue-bearing leaves did not affect the number of eggs laid by *Chilocorus nigrita*, but a complete, or near complete failure of eggs to hatch ensued when adults were exposed to either 3, 7 or 19 week old weathered residues from a single application of pyriproxyfen or triflumuron. Three week old residues of buprofezin had the same effect, but both 7 and 19 week old residues no longer significantly reduced egg viability. Adults of both *C. nigrita* and *Cryptolaemus montrouzieri* commenced laying viable eggs within 20 days of being separated from all residue-bearing leaves. One week old residues of pyriproxyfen and triflumuron both significantly reduced progeny production by *C. montrouzieri*. Ten week old triflumuron residues were still detrimental to this species but pyriproxyfen residues of the same age were not. It was concluded that IGRs are not compatible with integrated pest management (IPM) for citrus in southern Africa, where coccinellid biocontrol agents play an important role.

Introduction

The insect growth regulator (IGR) pyriproxyfen was first registered for the control of red scale *Aonidiella aurantii* (Maskell) (Homoptera: Diaspididae) on citrus in South Africa in 1991 and the product was first sprayed extensively in the Eastern Cape Province (Sundays River Valley). This was followed by a dramatic outbreak of cottony cushion scale *Icerya purchasi* Maskell (Homoptera: Margarodidae) by the end of that season. Populations of *Rodolia cardinalis* (Mulsant) (Coleoptera: Coccinellidae), the primary natural enemy of this pest in southern Africa, did not increase and extensive corrective organophosphate sprays were required to bring the pest under control. Subsequent use of pyriproxyfen in other areas of southern Africa did not lead to *I. purchasi* repercussions of the same magnitude as in the Eastern Cape. However, its use has been clearly associated with smaller repercussions on a widespread basis within southern Africa. Problems have mostly occurred in unsprayed orchards near to orchards which have received a pyriproxyfen treatment. Similar repercussions have been reported in Israel (Mendel & Blumberg, 1991; Mendel *et al.*, 1992).

Correspondence: Vaughan Hattingh, Outspan Citrus Centre, PO Box 28, Nelspruit 1200, Republic of South Africa.

The coccinellid *Chilocorus nigrita* (Fabricius) is an economically important predator of *A. aurantii* on citrus in the more humid, hotter regions of southern Africa. Nkwaleni Valley in northern Kwazulu/Natal is a highly suitable environment for *C. nigrita* and in the recent past, it could be found in large numbers playing an important role in the control of *A. aurantii* in this region. Since the commencement of extensive use of pyriproxyfen, *C. nigrita* has become extremely rare in Nkwaleni, even on giant bamboo *Dendrocalamus giganteus* (Gramineae) which previously served as an alternative habitat for large populations of the beetle. Likewise the population levels and efficacy of *C. nigrita* in Swaziland and the Eastern Transvaal lowveld have declined drastically since the commencement of pyriproxyfen use in these regions.

Numerous other coccinellids are important to varying degrees as biocontrol agents of many economically damaging insect pests on citrus, including mealybugs, phytophagous mites, armoured scales, soft scales and aphids. The detrimental effects of IGRs on coccinellids has only been reported in the literature on a limited scale (Peleg, 1983a; Loia & Viggiani, 1992) and they continue to be referred to as products which are compatible with integrated pest management (IPM). This, together with the field experience in southern Africa, made it clear that more information on the adverse effects of IGRs on coccinellids was needed to determine the extent to which their use can be incorporated into an IPM philosophy.

Chilocorus nigrita was chosen as an indicator species because of its economic importance in southern Africa and field experience which indicated that the use of IGRs was having a marked impact on their population levels. *Cryptolaemus montrouzieri* Mulsant (Coleoptera: Coccinellidae) was chosen as the other indicator species because of its economic importance internationally as a biocontrol agent of mealybugs. The bioassay technique used was not suitable for *R. cardinalis* which ceased ovipositing and quickly died on enclosure in the modified Munger cells. The adverse effects of the juvenile hormone analogue fenoxycarb (Insegar®) on *R. cardinalis* have already been demonstrated by Loia & Viggiani (1992).

The duration of toxicity of IGR residues under conditions of field-weathering is information which is critical to an assessment of the potential impact of IGRs on coccinellid populations, but is a topic which has not previously been investigated. Effects of exposure for various periods to field-weathered residues of three IGRs which are widely used in southern Africa, on the fecundity and egg viability of *Chilocorus nigrita* and *Cryptolaemus montrouzieri* were assessed.

Materials and methods

Individual trees in an experimental citrus orchard which had not been exposed to any plant protection products for the previous year, were each given a full cover spray of one of the following products: 30 ml pyriproxyfen 100 g a.i./l EC+300 ml Cipron narrow distillation range mineral oil/100 l water; 30 g buprofezin 500 g a.i./kg WP+500 ml Citrex mineral oil/100 l water; and 20 ml triflumuron 480 g a.i./l SC/100 l water. No subsequent sprays were applied to these trees. Leaves were picked at various times after application and placed inside modified Munger cells. The persistence of residues beyond 131 days could not be assessed because growth flushes which had occurred after spraying made it impossible to be sure that only sprayed leaves were being selected.

The modified Munger cells were similar to those described by Morse *et al.* (1986) and were constructed from 8 mm thick perspex blocks. A hole, 35 mm in diameter, was cut out of the centre of each block. A ventilation tunnel was drilled through opposite sides of the perspex block and fine stainless steel gauze placed over the cell openings to these tunnels. The cell was enclosed by two sheets of glass with a residue bearing leaf, on top of a wet sponge, clamped between one plate of glass and the bottom of the cell. Air was vented through the cells, via a plenum, at a rate of 1 volume exchange per minute.

An insectary culture of *C. montrouzieri* was maintained on citrus mealybug *Planococcus citri* (Risso) (Homoptera: Pseudococcidae) on butternuts *Cucurbita moschata* (Cucurbitaceae). Individual *Cryptolaemus montrouzieri* were sexed on the basis of the anterior legs of males being yellow and those of females being black (Fisher, 1963). A single pair was placed inside each cell together with a sliver of butternut with mealybug on it ($n=12$). The beetles laid their eggs on these butternut slivers which were replaced every second day together with the leaves.

Chilocorus nigrita were collected in citrus orchards which had not been exposed to any IGRs. These beetles were first maintained in the laboratory on oleander scale *Aspidiotus nerii* Bouché ((Homoptera: Diaspididae) on butternuts, to allow the adverse affects of a change in prey on their fecundity (Hattingh & Samways, 1992) to pass, before conducting the bioassay. Individual beetles were sexed according to the differences described by Samways & Tate (1984). Individual pairs were placed in each cell together with a sliver of butternut with *A. nerii* and a small pad of polyester fibre on which they oviposited ($n=15$). The leaves, egg pads and butternut slivers were replaced every second day.

Individual pairs of both species were each enclosed in the cells for 10 days. The egg pads and slivers of butternut, which were removed from the cells, were stored separately. On completion of 10 days of enclosure in the cells, the beetles were separated from the IGR residues and each species enclosed in a separate cage with clean food, and in the case of *C. nigrita*, with egg pads. The food and egg pads were replaced periodically and the egg hatch recorded.

Results

Exposure of *Cryptolaemus montrouzieri* to 7 and 70-day old triflumuron residues significantly reduced the number of larvae produced (table 1). Larval production was significantly reduced by exposure to a 7 day-old pyriproxyfen residue but no reduction was evident after 10 days of exposure to a 70 day-old residue. On completion of 10 days of exposure to the 70 day old residues, the number of larvae recovered from *C. montrouzieri* over 19 days of maintenance in a residue free environment, was not markedly reduced in the groups previously exposed to either of the IGRs.

Very few progeny were produced by *Chilocorus nigrita* during the first 2 days in the comparison of 21 day-old residues (table 2). This was probably due to commencement of the bioassay too soon after field collection and a forced change in prey species which is known to have a temporary deleterious influence on fecundity (Hattingh & Samways,

Table 1. Mean numbers of larvae recovered from pairs of *Cryptolaemus montrouzieri* after exposure for various periods to weathered IGR residues.

Product	Residue age (days)	Mean ± SE (*n*) larvae/pair/2 days from eggs laid during the exposure periods			Recovery period (days)	Total no. of larvae from 10 pairs over the recovery period
		0 to 2 d	4 to 6 d	8 to 10 d		
Control	–	$4.8^{a} \pm 1.3$ (12)	$3.5^{a} \pm 1.5$ (12)	$14.2^{a} \pm 2.5$ (11)	–	–
Triflumuron	7	$0.7^{b} \pm 0.3$ (12)	$0.5^{b} \pm 0.3$ (12)	$1.2^{b} \pm 1.2$ (11)	–	–
Pyriproxyfen	7	$2.9^{ab} \pm 1.1$ (12)	$0.3^{b} \pm 0.2$ (12)	$2.4^{b} \pm 1.3$ (11)	–	–
Control	–	–	–	$1.6^{a} \pm 0.5$ (10)	19	194
Triflumuron	70	–	–	$0.1^{b} \pm 0.1$ (10)	19	134
Pyriproxyfen	70	–	–	$1.9^{a} \pm 0.3$ (10)	19	159

A common letter in the same group (common residue age and exposure period) indicates no significant difference, Kruskal Wallis ANOVA, followed by a nonparametric multiple comparison, $\alpha = 0.05$ (Siegel & Castellan, 1989). Dosage rates are given in Materials and methods.

Table 2. Mean ± 1 SE (*n*) numbers of larvae recovered from pairs of *Chilocorus nigrita* after exposure for various periods to weathered IGR residues and percentage hatch of eggs laid after separation of adults from residues.

Product	Residues age (days)	Larvae/pair/2 days from eggs laid during the exposure periods			% hatch of eggs laid during the recovery periods			
		0 to 2 days	4 to 6 days	8 to 10 days	0 to 5 days	5 to 10 days	10 to 15 days	15 to 20 days
Control	–	$0.1^{a} \pm 0.1$ (15)	$1.6^{a} \pm 0.4$ (14)	$1.2^{a} \pm 0.6$ (13)	23	34	35	59
Buprofezin	21	$0.2^{a} \pm 0.1$ (15)	$0.1^{b} \pm 0.1$ (14)	$0^{b} \pm 0$ (14)	8	15	21	55
Triflumuron	21	$0.2^{a} \pm 0.2$ (15)	$0^{b} \pm 0$ (15)	$0^{b} \pm 0$ (15)	3	18	23	76
Pyriproxyfen	21	$0^{a} \pm 0$ (15)	$0^{b} \pm 0$ (15)	$0^{b} \pm 0$ (15)	0	0	0	24
Control	–	$2.9^{a} \pm 0.5$ (15)	$1.1^{a} \pm 0.5$ (15)	$0.7^{a} \pm 0.4$ (15)	70	88	80	85
Buprofezin	49	$2.5^{a} \pm 0.6$ (15)	$0.7^{ab} \pm 0.4$ (15)	$0.1^{a} \pm 0.1$ (15)	52	100	47	69
Triflumuron	49	$1.7^{a} \pm 0.6$ (15)	$0^{b} \pm 0$ (15)	$0.1^{a} \pm 0.1$ (15)	36	87	70	85
Pyriproxyfen	49	$0^{b} \pm 0$ (15)	$0^{b} \pm 0$ (15)	$0^{a} \pm 0$ (15)	0	0	6	53
Control	–	$3.0^{a} \pm 0.6$ (15)	$1.3^{a} \pm 0.3$ (15)	$1.1^{a} \pm 0.3$ (14)	66	92	32	65
Buprofezin	131	$1.9^{a} \pm 0.5$ (15)	$0.8^{ab} \pm 0.2$ (14)	$0.9^{a} \pm 0.3$ (14)	51	77	33	78
Triflumuron	131	$1.7^{a} \pm 0.5$ (15)	$0.2^{bc} \pm 0.2$ (14)	$0.2^{b} \pm 0.1$ (13)	37	93	65	59
Pyriproxyfen	131	$0.3^{b} \pm 0.2$ (15)	$0^{c} \pm 0$ (14)	$0^{b} \pm 0$ (14)	0	0	10	21

A common letter in the same group (common residue age and exposure period) indicates no significant difference, Kruskal Wallis ANOVA, followed by a nonparametric multiple comparison, $\alpha = 0.05$ (Siegel & Castellan, 1989). Dosage rates are given in Materials and methods.

Table 3. Mean ± 1 SE numbers of eggs/pair/2 days from *Chilocorus nigrita* after exposure for 8 to 10 days to weathered IGR residues.

Product	Residue age (days)	*n*	Eggs/pair/2 days
Control	–	13	7.8[a] ± 0.8
Buprofezin	21	14	7.1[a] ± 1.2
Triflumuron	21	15	7.7[a] ± 1.4
Pyriproxyfen	21	14	9.6[a] ± 1.4
Control	–	15	7.5[a] ± 1.0
Buprofezin	49	15	6.1[a] ± 1.0
Triflumuron	49	14	6.3[a] ± 0.9
Pyriproxyfen	49	14	4.8[a] ± 1.0
Control	–	14	4.4[a] ± 0.6
Buprofezin	131	14	3.2[a] ± 0.7
Triflumuron	131	13	5.2[a] ± 0.8
Pyriproxyfen	131	14	3.9[a] ± 0.8

A common letter in the same group (common residue age) indicates no significant difference, Kruskal Wallis ANOVA, $\alpha = 0.05$. Dosage rates are given in Materials and methods.

1992). There was a distinct decline in viability of control beetles towards the end of the next bioassay conducted with 49 day-old residues, precluding a meaningful comparison between treatments after 8-10 days exposure. This may have been due to inadvertent IGR contamination or advanced age of beetles in the culture. No results were obtained from a subsequent comparison of 84 day-old residues with beetles from this culture. The culture was replaced with fresh field material before undertaking a comparison of the 131 day old residues.

Exposure of adult *C. nigrita* to residue bearing leaves did not result in a significant reduction in the number of eggs laid (table 3). However, exposure of *C. nigrita* to pyriproxyfen residues after field weathering for 21 days, 49 days and 131 days, resulted in significant reductions in egg viability (table 2). A significant reduction in egg viability was evident after exposure for only 2 days to a 131 day-old pyriproxyfen residue, with complete elimination of egg viability after exposure for 6 days or longer.

Exposure of *C. nigrita* to triflumuron did not result in a significant reduction in egg viability as rapidly as when exposed to pyriproxyfen (table 2). There was however, a significant reduction in egg viability after 6 days or more of exposure to a 131 day-old triflumuron residue, with a mere 4% of eggs laid after 10 days of exposure being viable.

The detrimental effect of buprofezin on *C. nigrita* decreased with residue age more rapidly than in the case of pyriproxyfen or triflumuron (table 2). Although exposure to the 21 day old-residue for 6 days or more significantly reduced egg viability, such a detrimental effect was not evident after 49 days or 131 days of weathering.

Recovery in egg viability occurred rapidly after separation of the adults from the residue bearing leaves (table 2). The only compound with a marked persistence in the effect on egg viability after separation from the residue was pyriproxyfen. However, even these beetles started laying numerous viable eggs within 20 days of separation from the residue bearing leaves.

Discussion

The short duration of exposure to a residue contaminated substrate, required to induce sterility in coccinellids, was demonstrated by the extensive reduction in the viability of eggs laid by *C. nigrita* after only 48 h of exposure to a pyriproxyfen residue. Rapid uptake of pyriproxyfen has also been demonstrated with tsetse flies (Diptera: Glossinidae) where exposure for only one minute was sufficient to induce sterility (Langley *et al.*, 1990). However, the effect of adult exposure on the viability of coccinellid eggs was transient, since there was a rapid recovery in the ability of adults to lay viable eggs after their removal from the IGR contaminated environment. A similar reduced egg viability in nitidulids through exposure of adults to chitin synthesis inhibitors was demonstrated by Blumberg *et al.* (1985) and Ascher *et al.* (1986).

It is apparent from the results of this study that some variability exists in the extent of the adverse effects of different IGRs on a particular coccinellid species and in the effects of a particular IGR on different coccinellids. These results are a conservative indication of the adverse affects because many of the beetles were in direct contact with the leaf surface for only a small portion of the exposure time as much of the time was spent on the walls of the cells or on top of the slivers of butternut. The effect of ovipositing on a residue bearing surface and the effect of such residues on larval and pupal development were not assessed. According to the registration of pyriproxyfen in South Africa, it may be applied twice or three times a year, at approximately 42 day intervals, for the control of *A. aurantii* and buprofezin is often applied twice for mealybug control. Diofenolan (Aware) is another IGR which has recently been registered for use on citrus as a double spray. The residues of only single applications were tested in this study. The point at which the residues of pyriproxyfen and triflumuron are no longer detrimental to egg viability has not been established, but it is in excess of 131 days after a single application.

A particular orchard which may have received a number of IGR sprays for control of mealybug and *A. aurantii* during early- and mid-season, could be sprayed during late-season for false codling moth *Cryptophlebia leucotreta* (Meyrick) (Lepidoptera: Tortricidae). In such an orchard a viable population of *Chilocorus nigrita* could not exist. Considering the persistent effect of IGRs on this biocontrol agent, any use of an IGR can be expected to greatly impair the viability of the population for most if not all of the season in which it was used.

The persistence of IGR residues on citrus under field conditions and the extreme sensitivity of coccinellids to these residues, explains the repercussions of *I. purchasi* which have been experienced following the use of IGRs. However, the precise mechanism whereby the use of pyriproxyfen in one orchard disrupts the biocontrol of *I. purchasi* in a proximal unsprayed orchard, is not yet fully understood. Spray drift may be a plausible explanation. Alternatively, adults may become sterilized through contact with pyriproxyfen residues in a sprayed orchard prior to entering an unsprayed orchard, thereby reducing the ability of the coccinellid population to respond numerically to the prey population. However, if *Rodolia* spp. recover as rapidly as *C. nigrita* after removal from an IGR contaminated environment, this is unlikely to be the primary cause. Irrespective of the precise nature of disruption, because of the high degree of mobility displayed by these natural enemies, a patchwork of orchards contaminated with IGR residues can be expected to have an extensive impact on their regional population levels.

Often orchards in which pyriproxyfen has been sprayed do not experience an outbreak of *I. purchasi*, whereas repercussions are experienced in adjacent orchards which have not been sprayed. This may be attributed to the toxicity of pyriproxyfen to *I. purchasi* when the timing of application coincides with the synchronized occurrence of susceptible life stages. Pyriproxyfen is known to effectively control *I. purchasi* populations when applied at dosages of 50 or 100 ppm (Peleg, 1989), whereas it is registered for the control of *A. aurantii* in South Africa at 30 ppm.

It is surprising that IGRs, of the three chemical types used here, have been referred to in the literature as IPM compatible control measures (for example Hammann & Sirrenberg 1980; Blumberg *et al.*, 1985; Peleg, 1988; Ishaaya *et al.*, 1989; Ishaaya, 1990; Konno, 1990), when they are clearly highly detrimental to an important group of natural enemies. It is clear from these results that continued, frequent and widespread use of some IGRs precludes maximization of the biocontrol potential of economically important coccinellids in southern African citrus. However, the use of IGRs on citrus in southern Africa cannot simply be terminated, because they are also very effective control measures for important pests. It is important however, to recognize the extensive detrimental effect that they have on coccinellids and to limit their use as far as possible.

A number of IGRs have been shown not to have detrimental effects on hymenopteran parasitoids (Peleg, 1983b, 1988). If this holds true for all the most important hymenopteran parasitoids in citrus, the once-off use of IGRs could still be seen as a valuable tool in facilitating the transition from a chemical control approach to a bio-intensive IPM system. By bringing *A. aurantii* and mealybugs under control with IGRs, a viable hymenopteran parasitoid population may be maintained to ensure that subsequent abstinence from chemical intervention is not associated with economically damaging population outbreaks.

Alternative control measures which are compatible with IPM, such as mating disruption and augmentative releases of natural enemies, must still be developed for *Cryptophlebia leucotreta* to make it possible to dispense with IGRs for the control of this pest. In addition to the effects on insect natural enemies, further attention should also be given to the effect that IGR use may have on aquatic Crustacea through spray drift as they have been shown to be particularly sensitive to this group of products (Ishaaya, 1990).

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