In-Vitro Comparison of the Larvicidal Activity of Moxidectin and Abamectin Against *Onthophagus gazella* (F.) (Coleoptera: Scarabaeidae) and *Haematobia irritans exigua* De Meijere (Diptera: Muscidae)

W. M. DOHERTY[1], N. P. STEWART[1,2], R. M. COBB[3] and P. J. KEIRAN[4]

[1]Queensland Department of Primary Industries, P.O. Box 1085, Townsville Qld 4810.
[2]Present address: Queensland Department of Primary Industries, 665 Fairfield Rd, Yeerongpilly, Qld 4105.
[3]Cyanamid Australia, 5 Gibbon Rd., Baulkham Hills, N.S.W. 2153.
[4]Cyanamid International, 09-04/05 Fortune Centre, 190 Middle Rd, Singapore 0718.

ABSTRACT When incorporated directly into cattle dung, a formulation of moxidectin was less toxic to larvae of the dung beetle *Onthophagus gazella* and the buffalo fly, *Haematobia irritans exigua*, than an abamectin formulation. Concentrations of moxidectin 64-fold greater than abamectin concentrations were required to produce equivalent toxicities. Neither moxidectin nor abamectin reduced oviposition by *O. gazella*. Moxidectin may consequently be less likely than abamectin to affect the decomposition of cattle dung but may have less effect on buffalo fly infestations.

Introduction

Moxidectin is a milbemycin-like compound derived synthetically from nemadectin, a fermentation product of *Streptomyces cyaneogriseus noncyanogenus*. Like the avermectins abamectin and ivermectin, moxidectin is active against a wide range of nematodes and arthropods (Anon 1991; Webb *et al.* 1991). Moxidectin is being developed for commercial livestock applications similar to those of abamectin and ivermectin which are used to control internal and external parasites in livestock. The effects of such uses of avermectins on insects have been reviewed by Drummond (1985), Strong (1992) and Strong and Brown (1987). Regardless of treatment method avermectins are excreted almost entirely in the dung and primarily as the original compound (Jacob *et al.* 1983; Chiu and Lu 1989). When administered to cattle as single subcutaneous (SC) injections of 200 µg/kg liveweight, abamectin and ivermectin are excreted into the dung in concentrations which can be harmful to coprophagous insects for up to 5 weeks post-treatment (Miller *et al.* 1981; Schmidt 1983). Consequently, avermectins can decrease the rate of dung decomposition (Wall and Strong 1987; Madsen *et al.* 1990). However, other studies have found no effect on dung decomposition (Schmidt 1983; McKeand *et al.* 1988). Strong (1992) dismissed the studies which found no effect, generally because of methodological weaknesses. Moxidectin also has the potential to affect the dung fauna. When moxidectin was administered to cattle as slow-release intraruminal boluses, the dung subsequently produced was toxic to larvae of *Musca autumnalis* De Geer (Webb *et al.* 1991).

The coprophagous larvae of *Onthophagus gazella* (F.), an introduced species of dung beetle, and the buffalo fly, *Haematobia irritans exigua* De Meijere, have been shown to be sensitive to avermectins (Picton and Butler; Picton and Burrows; Burrows and Picton; unpublished results in Roncalli 1989). *O. gazella* is an important agent of bovine dung decomposition and *H. i. exigua* is a serious pest of cattle. Both species are widespread throughout northern Australia.

This study aimed to assess whether moxidectin may pose a threat to the dung fauna and hence dung decomposition in Australia and whether it may assist buffalo fly control.

Materials and methods

Injectable 1% formulations of moxidectin and abamectin were used because preliminary trials failed to find a solvent for technical grade moxidectin which was not toxic to the larvae of *H. i. exigua*.

Dung for both trials was obtained from a steer held in a slatted pen in an insect-proof enclosure. The animal was fed lucerne pellets *ad libitum*.

Haematobia irritans exigua. *H. i. exigua* were obtained from a laboratory colony established from flies collected at Townsville and maintained according to the methods of Thomas and Davis (1984) with the following modifications. The steer was fed lucerne pellets *ad libitum* and its dung was used as the larval medium. Flies were allowed to oviposit in the dung accumulated on a tray behind the steer. The dung was collected daily and left undisturbed for 24 h. It was then moistened if necessary to a moisture content of approximately 80%, formed into pats and placed on a 2 cm layer of sand. The dung was held a further 6 d at 26-30 °C after which pupae were retrieved from the sand by flotation.

Eggs were obtained by the method of Thomas and Davis (1984), washed into petri dishes and transferred onto damp filter paper.

Pats containing 4, 8, 16, 32, 64, 128, 256 and 512 µg of either moxidectin or abamectin/kg dung, were prepared by serial dilution of each formulation in dung. The concentrations tested were chosen to reflect the range of concentrations of moxidectin expected in dung following a single SC injection of 200 µg/kg liveweight, the standard treatment for cattle (unpublished Cyanamid data). The higher concentrations are likely in the days immediately after treatment with concentrations decreasing progressively through the lower concentrations on subsequent days. Each treatment was replicated five times. The highest concentration was prepared by adding formulation diluted in water to 1600 g of fresh dung and mixing for 30 min with a domestic food mixer. Five equal measures of dung, each approximately 150 g, were removed to provide the experimental pats. The remaining dung was reduced to 800 g, added to 800 g of fresh dung and mixed for a further 5 min to provide the second concentration. This procedure was repeated to provide the remaining treatments. Untreated control pats were prepared from fresh dung.

Each pat was placed on dry sand in a ventilated container. A batch of 100 eggs 2-6 h old was placed on each pat and the containers held at 25-30 °C for 7 d. Pupae were then harvested from the sand and the dung by flotation and held for 7 d at 27 °C after which adult eclosion was assessed.

Onthophagus gazella. Adults were collected 60 km NW of Townsville. A laboratory colony was established from eggs which had been collected from the brood balls produced by these adults, surface-sterilised and placed into artificial brood balls to avoid nematode contamination. The colony was maintained by the methods of Macqueen and Feehan (pers. comm.). Dung and soil used for the trial and the colony were treated to eliminate unidentified rhabdidiform nematodes, infestations of which appeared to reduce survival of adults and larvae and oviposition in previous colonisation attempts. Dung was frozen and thawed before use. Soil was autoclaved before use. Pats were similar to those in the *H. i. exigua* trial. However, the four highest concentrations of abamectin were excluded as preliminary trials had shown that no larvae survived at concentrations of 16 µg/kg dung or greater. Each treatment was replicated five times except the untreated control which was replicated 10 times.

Pats were placed on the surface of 3 kg of moist soil in a ventilated 4 L container. One pair of unmated beetles 5-7 d old was added to each container. Eight days later a further pair of unmated beetles aged 9-12 d was added to each container to increase the rate of dung burial. The containers were stored at 21-30 °C for the duration of the trial.

Fresh pats, prepared as previously described, were added to each container 3, 5, 8, 11 and 13 d after commencement to allow the beetles maximum opportunity for dung burial and oviposition. Any remnants of the previous pats were removed before the addition of new pats.

The soil, containing unburied dung and the

Table 1. Survival of larval and pupal *Haematobia irritans exigua* and oviposition and larval survival of *Onthophagus gazella* in dung treated with various concentrations of moxidectin and abamectin.

Treatment	Conc. µg/kg dung	*H. i. exigua*		*O. gazella*	
		Mean % Pupation (SE)	Mean % Eclosion (SE)	Mean No. Brood Balls (SE)	Mean % Survival to Adult (SE)
Moxidectin	4	38^{bc} (9.7)	99 (0.8)	48 (2.8)	89^{a} (4.1)
	8	55^{ab} (4.3)	98 (0.8)	49 (10.4)	81^{ab} (5.1)
	16	50^{ab} (6.5)	99 (0.7)	44 (2.7)	89^{a} (4.4)
	32	50^{ab} (9.0)	98 (1.0)	56 (5.1)	87^{a} (3.6)
	64	47^{ab} (5.3)	97 (0.9)	56 (6.4)	87^{a} (4.0)
	128	24^{cd} (3.2)	92 (3.4)	61 (6.1)	81^{ab} (3.2)
	256	13^{de} (1.8)	96 (2.5)	64 (1.8)	61^{cd} (5.3)
	512	0* (0)	na	70 (11.1)	7^{e} (2.5)
Abamectin	4	1^{e} (0.8)	83 (16.7)	42 (5.9)	57^{d} (6.8)
	8	0* (0)	na	53 (3.2)	5^{e} (1.9)
	16	0* (0)	na	44 (6.8)	0* (0)
	32	0* (0)	na	49 (6.0)	0* (0)
	64	0* (0)	na	nt	nt
	128	0* (0)	na	nt	nt
	256	0* (0)	na	nt	nt
	512	0* (0)	na	nt	nt
Untreated Control		62^{a} (8.2)	96 (1.4)	46 (6.2)	72^{bc} (4.2)

Means within each column with any similar adjacent letters are not significantly different (least significant difference at 5% level used).
* excluded from analysis.
na not applicable.
nt not tested.

beetles, was removed 18 d after commencement leaving only the brood balls in each container. A fresh soil layer was then added to each container. The numbers of brood balls, (each of which contained a single egg and hence was equivalent to oviposition), adults, pupae and larvae in each container were counted 53 d after commencement. Data were analysed by one-way analysis of variance. Treatments comprised entirely of zero values were excluded from analyses. Least significant differences (5% level) were calculated where a treatment effect was indicated.

Results

Moxidectin and abamectin affected both *H. i. exigua* and *O. gazella* (Table 1). Larval survival of *H. i. exigua* was reduced by all concentrations of abamectin and by concentrations of moxidectin of 128 μg/kg or greater. Survival was also reduced at 4 μg moxidectin/kg but in the absence of effects at other low concentrations this is unlikely to be a true treatment effect. Moxidectin at 256 and 512 μg/kg produced survival comparable to 4 and 8 μg/kg abamectin, respectively. Moxidectin did not affect the eclosion of adult *H. i. exigua*.

Neither moxidectin nor abamectin reduced oviposition by *O. gazella*. To the contrary, there was a consistent trend, although not significant, ($P = 0.10$), towards increased oviposition with increased concentrations of moxidectin. All concentrations of abamectin and 512 μg moxidectin/kg reduced larval survival of *O. gazella* with abamectin concentrations of 16 and 32 μg/kg producing complete mortality. As for *H. i. exigua*, moxidectin at 256 and 512 μg/kg produced survival comparable to 4 and 8 μg/kg abamectin respectively. Larval survival was actually increased at four of the five lowest concentrations of moxidectin.

Discussion

The injectable formulations of moxidectin and abamectin contained unknown and possibly dissimilar non-active ingredients, but the high dilution factors used would have reduced the likelihood of these affecting the trials.

Moxidectin displayed larvicidal activity against both *H. i. exigua* and *O. gazella*. However it was less toxic than abamectin for both species. Concentrations of moxidectin 64-fold greater than abamectin concentrations were required to produce similar effects.

Avermectins excreted in dung have demonstrated adverse effects on oviposition by other species of scarabeine dung beetles (Ridsdill-Smith 1988; Wardhaugh and Rodriguez-Menendez 1988; Houlding *et al.* 1991). These effects are generally limited to immature adult beetles (Strong 1992). No effects were detected for either moxidectin or abamectin on the mature adult *O. gazella* used in this trial and consequently dung burial was also unaffected.

It is difficult to explain the apparent beneficial effects of the lower concentrations of moxidectin on survival of larval *O. gazella* and the trend towards increased oviposition at higher concentrations of moxidectin. Although the nematode infestations mentioned previously were minimised in the dung and soil used both in the experiment and the colony to rear *O. gazella*, their presence in the experiment in low numbers remains a possibility. It is possible that moxidectin killed these nematodes and consequently enhanced larval survival and adult fecundity. Wardhaugh and Mahon (1991) found that dung from abamectin-treated cattle attracted more scarabeine dung beetles than dung from untreated animals and that beetles remained in the treated dung for longer than the untreated. They suggested that volatile metabolites of the abamectin formulation or its effects on the microbial flora of the dung may have been responsible for these effects. Such possibilities could also be responsible for the unexplained effects of moxidectin in this trial.

Since our study was conducted, an *in-vivo* trial of moxidectin against *O. gazella* and another dung beetle, *Euoniticellus intermedius* (Reiche), has been published (Fincher and Wang 1992). Dung was collected from cattle up to 42 d after each received a single moxidectin injection of 200 μg/kg. Oviposition and larval survival were neither reduced nor enhanced at any time in either species. Thus it appears that under normal use moxidectin concentrations will remain below harmful levels for *O. gazella* and possibly *H. i. exigua*. Burrows and Picton (unpublished results in Roncalli 1989) recorded complete mortality of *H. i. exigua* and *O. gazella* larvae in dung collected up to 21 d after treatment of cattle with a single SC injection of 200 μg/kg abamectin. Consequently moxidectin may be less likely than abamectin to hamper dung decomposition but may also contribute less to buffalo fly control.

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