



The combined mode of action of fipronil and amitraz on the motility of *Rhipicephalus sanguineus*

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ABSTRACT

The motility of adult *Rhipicephalus sanguineus* was evaluated subsequent to treatments of amitraz, fipronil and the combination of fipronil plus amitraz against a vehicle control in a Petri dish assay using the LemnaTec Scanalyzer Imaging System. The assay was run using a fixed dilution of amitraz (0.32 µg/cm²); serial dilutions of fipronil (1.3, 0.33, 0.08, 0.02, or 0.005 µg/cm²); and the same serial dilutions of fipronil in combination with the fixed dilution of amitraz. Measurement of motility was made of unstimulated ticks and then after stimulation at 1, 4, 18–22, and 24 h post exposure (hpe) of the Petri dishes. For the unstimulated ticks, there was no difference in motility between the amitraz treatment group and the fipronil plus amitraz treatment group at the early time points. However, these two treatment groups had significantly higher motility than the solvent control and fipronil treatment groups. The unstimulated ticks in the amitraz treatment group had significantly higher motility than the fipronil plus amitraz treatment group at the later time points. Measurements after stimulation demonstrated there was no difference in motility between the amitraz treatment group and the fipronil plus amitraz treatment group at the early time points. By 18 hpe, the fipronil plus amitraz treatment group had significantly lower motility than all other treatment groups and at 21–22 and 24 hpe the other treatment groups did not differ from the control group. The action could be divided in two phases in the combination experiment: *phase 1*: an early increase in motility due to amitraz is identified in both amitraz alone or fipronil plus amitraz groups; *phase 2*: the combination of fipronil plus amitraz caused a significantly greater reduction in motility, suggesting mortality of the ticks, compared to fipronil or amitraz alone. These results demonstrate a synergism resulting from the combination of fipronil plus amitraz.

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1. Introduction

Fipronil and amitraz (Fig. 1) have a long history of use in dogs. As active pharmaceutical ingredients, fipronil and amitraz each target specific receptor types in fleas and/or ticks, modulating these to achieve the efficacy

of the product. Although there is also some activity on mammalian receptors, the specificity to parasite receptors allows for a margin of safety for dogs receiving treatment with these agents.

1.1. Fipronil binding to invertebrate GABA- and glutamate-gated chloride channels

Fipronil binds to an agonist-bound form of the gamma-aminobutyric acid (GABA) receptors in the cell membranes

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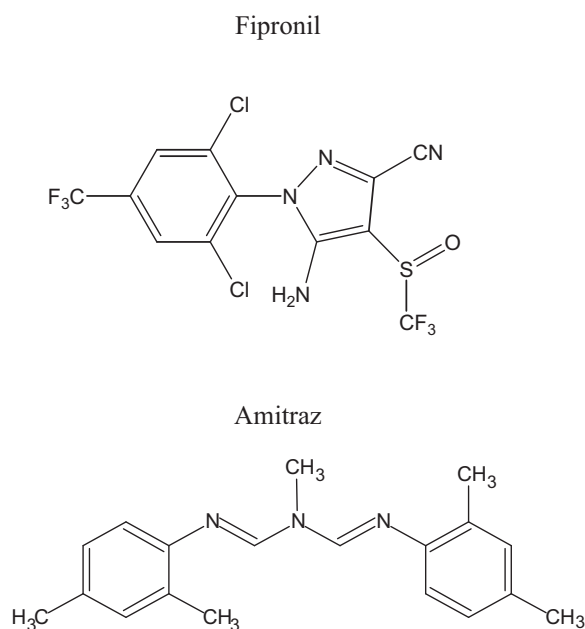


Fig. 1. Chemical structures of fipronil and amitraz.

of invertebrate neurons, functionally stabilizing the closed form of the channel and blocking the GABA-mediated inhibitory action that results from the inward movement of chloride ions across these membranes (illustrated in Fig. 2) (Zhang and Jackson, 1993). Additionally, fipronil and its metabolite fipronil sulfone block two types of glutamate-activated chloride channels in insects (Zhao et al., 2005). These receptors are found in invertebrates, but not in vertebrates (Raymond and Sattelle, 2002; Zheng et al., 2003; Zhao et al., 2005). The decrease in inhibitory modulation of neuronal action potentials results in seizures and seizure-like behaviors, and death in target parasitic species (Aidley, 1996).

1.2. Amitraz acts at the tick octopamine receptor

The probable mode of action of amitraz as an acaricide results from agonism of octopamine receptors of ectoparasites. The function of octopamine in invertebrates is similar to that of the adrenergic system in vertebrates (Roeder, 1999). Octopamine is widely distributed in insect tissues,

and mediates its actions through three receptor classes (Evans and Maqueira, 2005). These classes are designated α -adrenergic-like, β -adrenergic-like, and octopaminergic/tyraminergeric receptors. Octopamine receptors are coupled to G-proteins that are linked to adenylate cyclase (Nathanson, 1985; Roeder, 1995). In insects, octopamine functions as: (1) a neurotransmitter, controlling endocrine gland activity; (2) a neurohormone, inducing mobilization of lipids and carbohydrates; (3) a peripheral neuromodulator, acting on muscles, the fat body, corpora cardiaca and corpora allata; and (4) a central neuromodulator influencing motor patterns, habituation and memory (Evans, 1981; Hirashima and Huang, 2008). The overstimulation of octopamine synapses in the CNS results in tremors and convulsions in juvenile and adult arthropods. At sublethal levels these compounds influence egg-laying, feeding activity and raise the excitatory state of the arthropod (Orr et al., 1990). The effects of amitraz on octopamine receptors of arthropods are illustrated (Fig. 3).

Booth (1989) characterized the effects of octopaminergic and adrenergic drugs on oviposition in *Rhipicephalus microplus* (formerly *Boophilus microplus*). Octopamine and amitraz inhibited oviposition in this tick as did the α -adrenergic agonists clonidine and naphazoline. β -Adrenergic agonists and antagonists, histamine receptor agonists, dopamine and serotonin had no effect. Curiously, synerphine and apomorphine, which activate octopamine receptors in insects, had no effect on oviposition. The lack of synerphine and apomorphine effects on oviposition could be explained by the differences in the known tick and insect octopamine receptors. Taken together, these results strongly suggest that the effects of amitraz on ticks result from octopamine receptor activation.

Baxter and Barker (1999) identified a gene for a putative octopamine receptor in an Australian strain of the tick *R. microplus* that showed 60–70% sequence homology with known insect octopamine receptors. The latter gene was subsequently compared between known amitraz resistant and susceptible strains of *R. microplus* in the Americas, and amino acid substitutions were consistently identified at two loci in the resistant strains (Chen et al., 2007). At this time the study by Chen et al. (2007) provides the primary evidence that amitraz is acting on octopamine receptors in ticks.

Because fipronil and amitraz bind at different receptor sites it was expected that these multiple sites of action would provide increased activity against the tick in com-

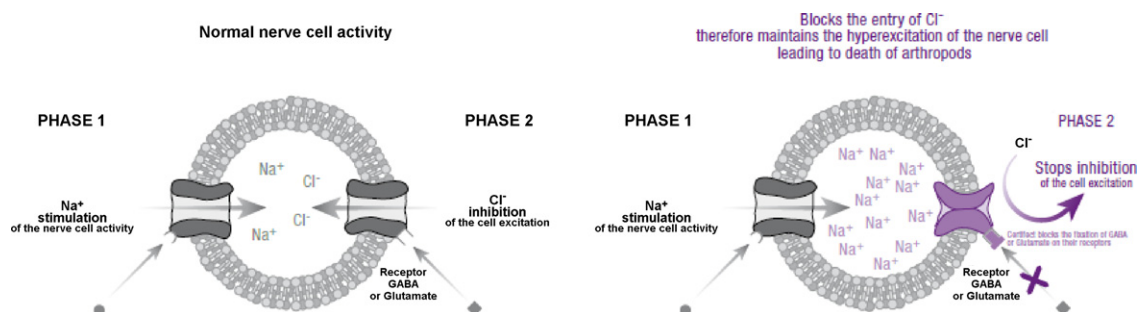


Fig. 2. Functional consequences of fipronil binding to invertebrate GABA or glutamate receptors.

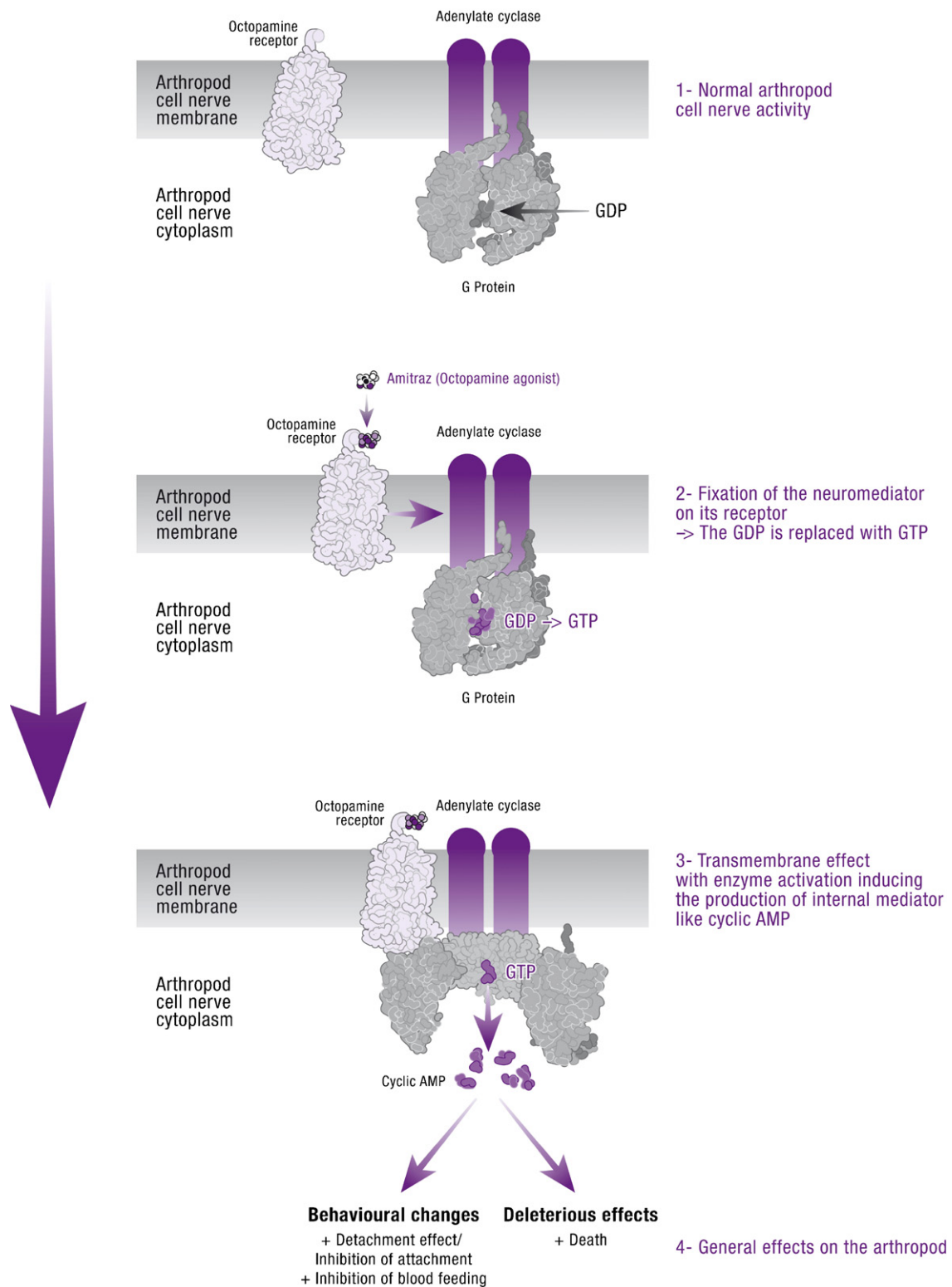


Fig. 3. Functional consequences of amitraz binding to the tick octopamine receptor.

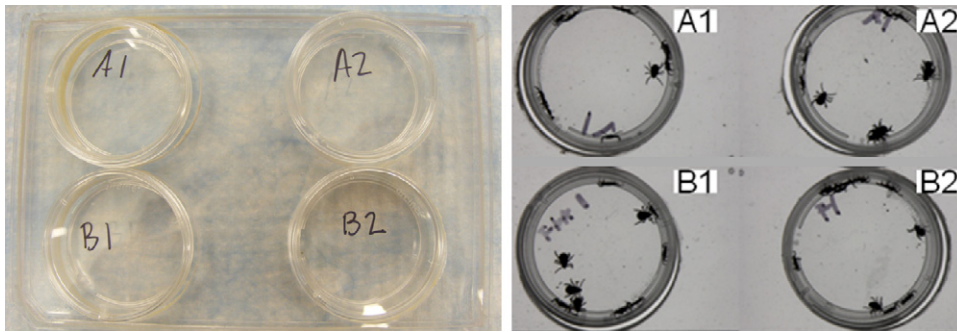


Fig. 4. Plate for holding the 4 Petri dishes for evaluation using the LemnaTec Scanalyzer system.

parison with one agent alone. Fipronil and amitraz are formulated in a novel combination, CERTIFECT™ (Merial Limited, GA, USA), that provides a robust tick treatment, both in terms of speed of kill and duration of effect (Baker et al., 2011; Hunter et al., 2011). The objective of this study was to investigate the effect of the combination of the two acaricidal molecules of the combination on the motility and mortality of ticks over time and to investigate a potential mode of action for the synergistic effect.

2. Materials and methods

2.1. Experimental procedure

The LemnaTec Scanalyzer system is designed to capture digital images of a subject and quantify aspects of those images. In this application the system was set up to capture multiple images of a plate holding four Petri dishes and compare those images to quantify movement within the dishes.

A plate holding the four Petri dishes was custom made to standardize the position of the dishes on a lightbox (Fig. 4). Dish A1 was treated with ethanol as a solvent control. Dish A2 was treated with amitraz at a constant rate of $0.32 \mu\text{g}/\text{cm}^2$ for all assays. Dish B1 was a combination of fipronil and amitraz. The amitraz was applied at $0.32 \mu\text{g}/\text{cm}^2$ and fipronil was serially diluted to make solutions to be applied at five doses of 1.3, 0.33, 0.08, 0.02, or $0.005 \mu\text{g}/\text{cm}^2$. Dish B2 was treated with fipronil at the same dose as used to treat Dish B1. This resulted in 5 plates being evaluated at each time point with 1 of the 5 fipronil doses applied to Dishes B1 and B2 in each of the 5 plates.

A stock 10,000 ppm solution of fipronil (analytical standard, CAS No. 120068-37-3, Sigma–Aldrich, Inc.) and amitraz (analytical standard, CAS No. 33089-61-1, Sigma–Aldrich, Inc.) were formulated in ethanol (absolute, 200 proof $\geq 99.5\%$ A.C.S. reagent, CAS No. 64-17-5, VWR) at a rate of $100 \mu\text{L}/\text{mg}$ of compound. A volume of $10 \mu\text{L}$ each from the fipronil and amitraz stock solutions was pipetted each into $3990 \mu\text{L}$ of ethanol to make a 25 ppm solution with a total volume of 4 mL.

For Dish A1, the top and bottom portions of the Petri dishes were treated with $625 \mu\text{L}$ of ethanol each (all Petri dishes were 35 mm in diameter). For Dish A2, the top and bottom portions of the Petri dishes were treated with $125 \mu\text{L}$ of the 25 ppm amitraz solution each. For Dish B1,

the top and bottom portions of the Petri dishes were treated with $500 \mu\text{L}$ of the 25 ppm solution of fipronil each (serially diluted for each Plate 1–5) and separately with $125 \mu\text{L}$ of the 25 ppm solution of amitraz. For Dish B2, the top and bottom portions of the Petri dishes were treated with $500 \mu\text{L}$ of the 25 ppm solution of fipronil each (serially diluted for each Plate 1–5). The Petri dishes were carefully tilted to distribute the ethanol or solution over their surfaces.

The Petri dishes were left open under a fan and allowed to dry for 1 h. After drying, 10 adult *Rhipicephalus sanguineus* (supplied by Ecto Services, Inc., Henderson, NC) were put in the dishes. Each dish was secured by wrapping parafilm around the edge with air holes punched into the parafilm for air exchange. The ticks were left undisturbed for 1 h post-exposure (hpe) before the initial evaluation in the LemnaTec Scanalyzer system. Between evaluations ticks were maintained at approximately 21°C and 60% RH.

After the initial hour each Plate, 1–5, was sequentially placed into the Scanalyzer for evaluation. The camera captured ten images 10 ms apart for each Petri dish. Image processing software calculated the change in movement of ticks between each image. The value generated is equal to the raw movement in each Petri dish during the image time. The ticks in each individual Petri dish were then stimulated to move by breathing/exhaling into the plate. After stimulation, the plates were placed back into the Scanalyzer for another evaluation to determine if the treatment affected the tick's ability to move. Image capture was repeated as in the pre-stimulated evaluation.

Further evaluations were performed for a total of 4 time points post-exposure to the treatments. Six independent trials were conducted. The 1 hpe evaluation was performed in all 6 trials. A 4 hpe evaluation was added in Trials 4 through 6 to further characterize the early changes in motility caused by the treatments. The third evaluation was performed at 18 hpe in Trials 1 through 3 and at 21–22 hpe in Trials 4 through 6 for logistical reasons. A 24 hpe evaluation was performed in all six trials.

2.2. Data analysis

Two statistical evaluations were performed. The first compared the treatment effects, while the second examined the effect of the doses. Both evaluations were conducted separately within each time point and for unstimulated and stimulated ticks.

For each pairwise comparison of treatments in the first evaluation, both the comparison of the main treatment effects as well as the interaction between the two treatments and the fipronil doses were made. When there was no evidence of interaction between the treatments and the doses, the comparisons of the main effects provided a complete summary of the difference between the treatments. When there was evidence of treatment-by-dose interaction, this generally meant one of two things, depending upon whether or not the main effect was statistically significant. If the main effect was statistically significant, then either Treatment A was significantly greater than Treatment B for every dose, but at different significance levels, or Treatment A was significantly greater than Treatment B at some of the doses, and the two treatments were not significantly different for the rest. In either case, Treatment A was significantly greater than Treatment B at multiple doses. For the case where the main effect was not statistically significant, Treatment A was significantly greater than Treatment B for some doses, while Treatment B was significantly greater than Treatment A for other doses. For the purposes of this analysis, this situation will be interpreted as if there was no evidence of difference between the two treatments.

To determine whether the two fipronil treatments had a dose effect in the second evaluation, the global statistic was used that tested the null hypothesis that all the doses had equal means within a treatment versus the alternative hypothesis that at least two dose means differed. Note that the analysis stopped at this level, and no separation of doses within a treatment was undertaken.

The natural logarithm plus 1 of the unstimulated and stimulated tick assay results (raw movement value calculated by Image processing software) was analyzed within each measurement time using a general linear mixed model. For each measurement time, the Mixed procedure in SAS Version 9.1.3 was used with treatment, fipronil dose, and its interaction as the fixed effects, and trial and the fipronil dose-by-trial interaction as the random effects. The analysis of the logarithm of the assay responses meant that summaries used the geometric mean. The model and subsequent analysis evaluated each treatment group at each fipronil dose at each measurement time.

All statistical hypotheses were tested at the (two-sided) $\alpha = 0.05$ significance level.

3. Results

Normally adult *R. sanguineus* will rest unless there is a source of stimulation, such as movement or CO₂. The fipronil treated ticks and the ethanol solvent control ticks displayed this behavior which was captured as a low motility value by the imaging system in the unstimulated evaluations.

For the unstimulated ticks, at 1 and 4 hpe there was no difference in motility when compared over all fipronil doses between the amitraz treatment group and the fipronil plus amitraz treatment group. However, these two treatment groups had a significantly higher motility than the solvent control and fipronil treatment groups. At 18, 21–22, and 24 hpe the amitraz treatment group had significantly

Table 1

Analysis of treatment effects by hour for unstimulated ticks in the LemaTec Scanalyzer assay.^{a,b}

Hours post exposure	Treatment group	Geometric mean	Pairwise comparisons ^c
1	Control	1760	A
1	Amitraz	18,900	B
1	Fipronil + amitraz	27,800	B
1	Fipronil	2170	A
4	Control	258	A
4	Amitraz	26,300	B
4	Fipronil + amitraz	32,300	B
4	Fipronil	1600	A
18	Control	104	A
18	Amitraz	35,100	B
18	Fipronil + amitraz	3300	C
18	Fipronil	459	A–C
21–22	Control	179	A
21–22	Amitraz	27,700	B
21–22	Fipronil + amitraz	1620	C
21–22	Fipronil	767	A–C
24	Control	518	A
24	Amitraz	31,200	B
24	Fipronil + amitraz	1090	A
24	Fipronil	1720	A

^a The logarithm of the motility (measurements + 1) was analyzed as a general linear mixed model at each time point using the Mixed procedure in SAS Version 9.1.3. The factors dose, treatment, and their interaction were the fixed effects, and trial and the trial-by-dose interaction were the random effects.

^b Within each time point, the overall treatment effects were compared on a pairwise basis taking into account both main treatment effect as well as the treatment-by-dose interactions.

^c Treatments sharing same letter are not significantly different at the $\alpha = 0.05$ level.

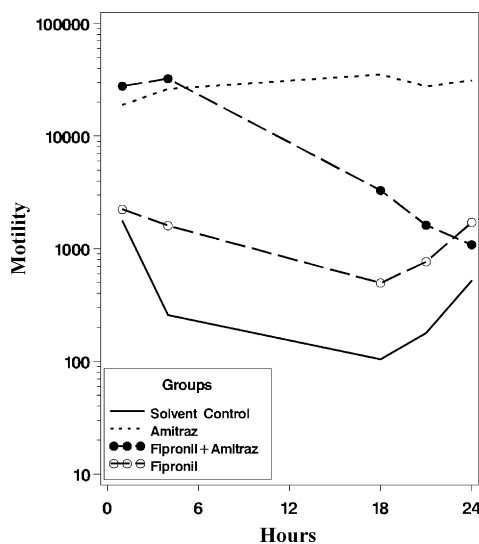


Fig. 5. Geometric means of motility assay calculated over all doses for unstimulated ticks through time.

cantly higher motility than the fipronil plus amitraz group. Solvent controls and the fipronil treatment groups were in a state of rest with low motility values at the 18 and 21–22 hpe evaluation. At 24 hpe, there was no difference between solvent control, fipronil, and fipronil plus amitraz treatments (Table 1 and Fig. 5).

Table 2Analysis of treatment effects by hour for stimulated ticks in the LemnaTec Scanalyzer assay.^{a,b}

Hours post exposure	Treatment group	Geometric mean	Pairwise comparisons ^c
1	Control	43,300	A
1	Amitraz	56,900	B
1	Fipronil + amitraz	59,400	B
1	Fipronil	51,000	A, B
4	Control	30,600	A, B
4	Amitraz	44,300	C
4	Fipronil + amitraz	38,500	A, C
4	Fipronil	29,000	B
18	Control	30,400	A, B
18	Amitraz	33,200	A
18	Fipronil + amitraz	3700	C
18	Fipronil	17,400	B
21–22	Control	25,000	A
21–22	Amitraz	30,300	A
21–22	Fipronil + amitraz	1990	B
21–22	Fipronil	21,200	A
24	Control	27,600	A, B
24	Amitraz	34,300	A
24	Fipronil + amitraz	598	C
24	Fipronil	19,700	B

^a The logarithm of the motility (measurements + 1) was analyzed as a general linear mixed model at each time point using the Mixed procedure in SAS Version 9.1.3. The factors dose, treatment, and their interaction were the fixed effects, and trial and the trial-by-dose interaction were the random effects.

^b Within each time point, the overall treatment effects were compared on a pairwise basis taking into account both main treatment effect as well as the treatment-by-dose interactions.

^c Treatments sharing same letter are not significantly different at the $\alpha = 0.05$ level.

For stimulated ticks, at 1 and 4 hpe there was no difference in motility when compared over all fipronil doses between the amitraz treatment group and the fipronil plus amitraz treatment group. At 18, 21–22, and 24 hpe, the fipronil plus amitraz treatment group had significantly lower motility than all other treatment groups and the other treatment groups did not differ from the control group (Table 2 and Fig. 6).

The analysis of the fipronil dose for the fipronil and fipronil plus amitraz treatments for unstimulated ticks demonstrated there was a significant difference in motility between doses for the fipronil plus amitraz treatment at every measurement time. However, for the fipronil alone treatment there was no evidence that means of motility measurements changed over the doses (Table 3).

From the analysis of the effect of the fipronil dose for stimulated ticks the fipronil treatment group had significantly different mean motility between at least 2 doses at 18 and 24 h. The fipronil plus amitraz treatment group had significantly different mean motility between doses at 4, 18, 21–22, and 24 h (Table 4). Fig. 7a through e illustrates the effect of the fipronil plus amitraz combination over time. At 18 and 21–22 hpe, doses as low as 0.08 $\mu\text{g}/\text{cm}^2$ of fipronil in the fipronil plus amitraz combination showed reductions in motility. The ticks in the fipronil only treatment group were not affected in this manner. For the 18, 21–22, and 24 hpe evaluations, only the highest dose of fipronil (1.3 $\mu\text{g}/\text{cm}^2$) had consistently low motility values after stimulation (Fig. 7c–e). This greater reduction in

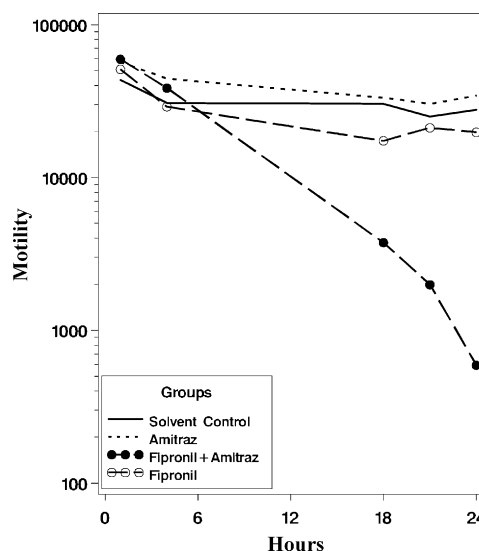


Fig. 6. Geometric means of motility assay calculated over all doses for stimulated ticks through time.

motility for fipronil plus amitraz was also measured at the 24 hpe time point down to the 0.02 $\mu\text{g}/\text{cm}^2$ dose of fipronil tested (Fig. 7e).

4. Discussion

Dawkins and Gladney (1978) reported that amitraz caused detachment and hyperactivity in various species of ticks. The present study demonstrated that amitraz does cause increased motility in *R. sanguineus* similar to that previously reported and seen in insects exposed to amitraz. In the present study the significantly greater motility of ticks in the amitraz and fipronil plus amitraz treatment groups at 1 and 4 hpe over control and fipronil treatment groups demonstrates that treatments with amitraz had the effect

Table 3Comparison of mean motility measurements within treatment groups for unstimulated ticks to test that doses of fipronil are different.^a

Hour post exposure	Treatment analyzed	p-Value ^b
1	Fipronil + amitraz	0.0373
1	Fipronil	0.5738
4	Fipronil + amitraz	0.0333
4	Fipronil	0.3846
18	Fipronil + amitraz	0.0157
18	Fipronil	0.7256
21	Fipronil + amitraz	0.0097
21	Fipronil	0.2706
24	Fipronil + amitraz	0.0003
24	Fipronil	0.3426

^a The logarithm of the motility measurements + 1 was analyzed as a general linear mixed model at each time point using the Mixed procedure in SAS Version 9.1.3. The factors dose, treatment, and their interaction were the fixed effects, and trial and the trial-by-dose interaction were the random effects. The results in this table are associated with an F-test with 4 numerator degrees of freedom.

^b Two-sided p-values testing the null hypothesis that the means of all doses are equal versus the alternative that at least two of the means of the doses within the listed treatments are different.

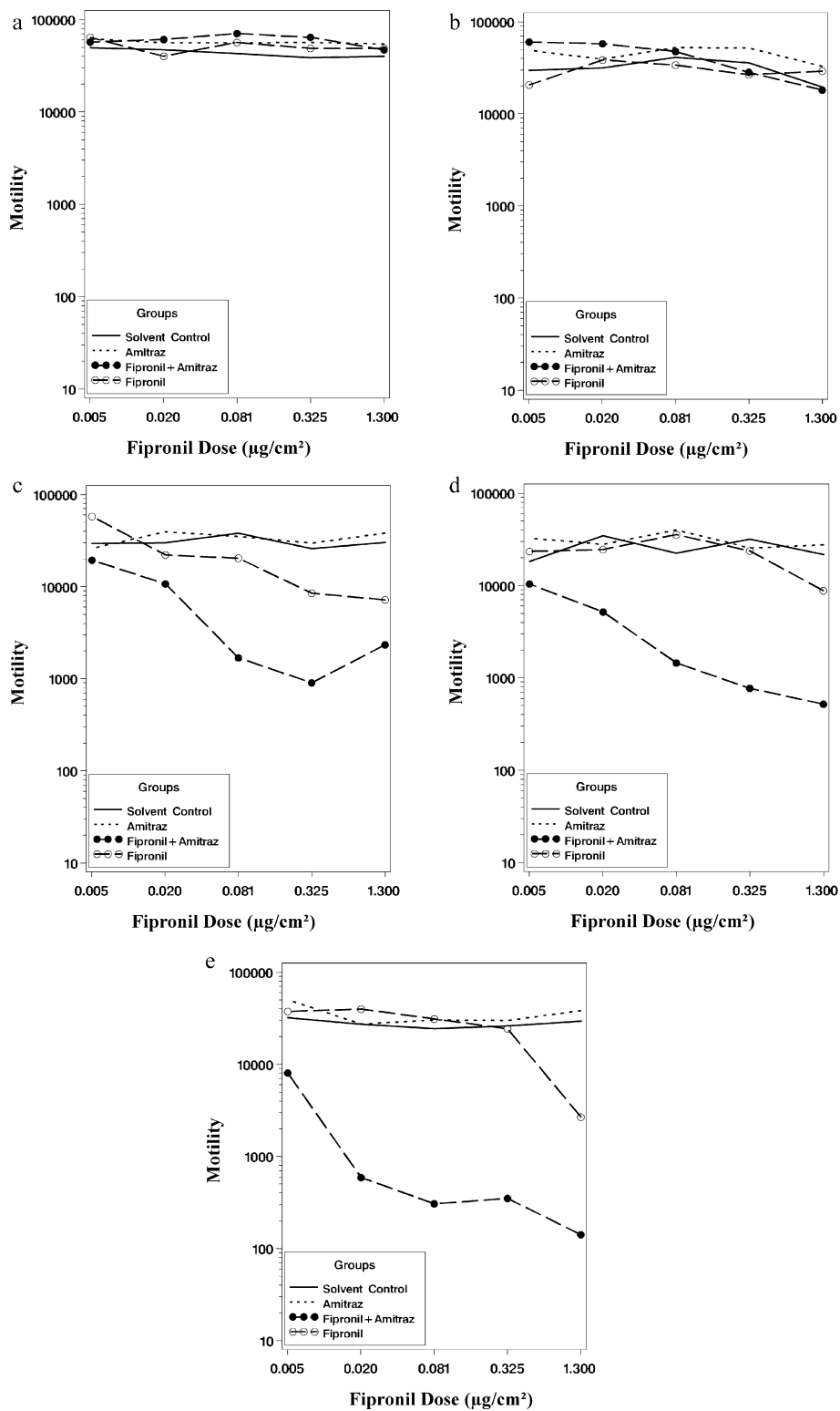


Fig. 7. (a) Geometric means of motility assay across fipronil doses for stimulated ticks at hour 1. (b) Geometric means of motility assay across fipronil doses for stimulated ticks at hour 4. (c) Geometric means of motility assay across fipronil doses for stimulated ticks at hour 18. (d) Geometric means of motility assay across fipronil doses for stimulated ticks at hour 21–22. (e) Geometric means of motility assay across fipronil doses for stimulated ticks at hour 24.

Table 4

Comparison of mean motility measurements within treatment groups for stimulated ticks to test that doses of fipronil are different.^a

Hour post exposure	Treatment analyzed	p-Value ^b
1	Fipronil + amitraz	0.2266
1	Fipronil	0.1790
4	Fipronil + amitraz	0.0023
4	Fipronil	0.3772
18	Fipronil + amitraz	<0.0001
18	Fipronil	0.0318
21	Fipronil + amitraz	0.0013
21	Fipronil	0.3471
24	Fipronil + amitraz	0.0053
24	Fipronil	0.0004

^a The logarithm of the motility measurements + 1 was analyzed as a general linear mixed model at each time point using the Mixed procedure in SAS Version 9.1.3. The factors dose, treatment, and their interaction were the fixed effects, and trial and the trial-by-dose interaction were the random effects. The results in this table are associated with a *F*-test with 4 numerator degrees of freedom.

^b Two-sided *p*-values testing the null hypothesis that the means of all doses are equal versus the alternative that at least two of the means of the doses within the listed treatments are different.

of increasing the motility of the ticks at these early time points. When measuring the motility of stimulated ticks from 18 hpe to 24 hpe for the fipronil plus amitraz treatment group there was significantly less movement than the other 3 treatment groups. This demonstrates that the fipronil plus amitraz treatment has a greater effect on the motility of stimulated ticks than treatments with fipronil or amitraz alone. In evaluations of tick mortality, lack of movement in a stimulated tick is a key factor in determining the live/dead status of the tick (Dryden et al., 2006; Rugg and Hair, 2007). Therefore, the lack of movement also indicates an increase in mortality of ticks treated with fipronil plus amitraz over treatments of fipronil or amitraz alone. Additionally, the difference in the dose response between the fipronil alone group and fipronil plus amitraz group illustrated in Fig. 7a through e demonstrate that a lower amount of fipronil was adequate in combination with amitraz to cause mortality at the later time points.

Physiologic mechanisms have been proposed for the synergism of pesticides by amitraz including, modifying binding of pyrethroids to the receptor site on insect chloride channels, or reducing the degradation of pesticides by arthropods (Liu and Plapp, 1992; Usmani and Knowles, 2001). Treacy et al. (1987) hypothesized that it was the increase in movement of *Heliothis virescens* on plants treated with chlordimeform, a formamidine pesticide similar to amitraz, that resulted in increased exposure to pyrethroids applied concurrently and resulted in synergism. The proposed mechanism was adhesion of the pesticide to the insect as it moved over treated surfaces. Our data would support the hypothesis of Treacy et al. (1987) and support extension of this potential mechanism to ticks as demonstrated by the early increase in motility of the ticks exposed to amitraz leading to a greater exposure of the ticks to fipronil in the fipronil plus amitraz combination. The combined mode of action could be divided into two steps: step one due to amitraz, characterized by an increase in motility and inducing an increased exposure to fipronil; and step two due to fipronil, characterized

by a greater mortality of ticks at later time points than observed in either active alone. In earlier work, synergism and improved speed-of-kill for the combination of amitraz and fipronil was demonstrated against *R. sanguineus* (Prullage et al., 2011). The current study provides further evidence for the synergism of the fipronil and amitraz combination and suggests a possible mechanism of action.

Conflict of interest

The work reported herein was partly funded by Merial Limited, GA, USA.

Three authors (JBP, JH and STC) are current employees of Merial, and assisted with study design, data analysis and review of the manuscript, however there were no conflicting interests that may have biased the work reported in this paper.

References

- Aidley, D., 1996. In: Aidley, D., Stanfield, P. (Eds.), *Ion Channels*. Cambridge University Press, Cambridge.
- Baker, C.F., Hunter III, J.S., McCall, J.W., Young, D.R., Hair, J.A., Everett, W.R., Yoon, S.S., Irwin, J.P., Young, S.L., Cramer, L.G., Pollmeier, M.G., Prullage, J.B., 2011. Efficacy of a novel topical combination of fipronil, (S)-methoprene and amitraz for treatment and control of induced infestations with North American ticks *Dermacentor variabilis*, *Ixodes scapularis*, *Amblyomma americanum* and *Amblyomma maculatum*. *Vet. Parasitol.* 179, 318–323.
- Baxter, G.D., Barker, S.C., 1999. Isolation of a cDNA for an octopamine-like, G-protein coupled receptor from the cattle tick, *Boophilus microplus*. *Insect Biochem. Mol. Biol.* 29, 461–467.
- Booth, T.F., 1989. Effects of biogenic amines and adrenergic drugs on oviposition in the cattle tick *Boophilus*: evidence for octopaminergic innervation of the oviduct. *Exp. Appl. Acarol.* 7, 259–266.
- Chen, A.C., He, H., Davey, R.B., 2007. Mutations in a putative octopamine receptor gene in amitraz-resistant cattle ticks. *Vet. Parasitol.* 148, 379–383.
- Dawkins, C.C., Gladney, W.J., 1978. Detachment of four species of ticks exposed to selected formamidine compounds. *J. Econ. Entomol.* 71, 657–660.
- Dryden, M.W., Payne, P.A., Smith, V., Hostetler, J., 2006. Evaluation of an imidacloprid (8.8% w/w)–permethrin (44.0% w/w) topical spot-on and a fipronil (9.8% w/w)–(S)-methoprene (8.8% w/w) topical spot-on to repel, prevent attachment, and kill adult *Ixodes scapularis* and *Amblyomma americanum* ticks on dogs. *Vet. Ther.* 7, 173–186.
- Evans, P.D., 1981. Multiple receptor types for octopamine in the locust. *J. Physiol.* 318, 99–122.
- Evans, P.D., Maqueira, B., 2005. Insect octopamine receptors: a new classification scheme based on studies of cloned *Drosophila* G-protein coupled receptors. *Invert. Neurosci.* 5, 111–118.
- Hirashima, A., Huang, H., 2008. Homology modeling agonist binding site identification, and docking in octopamine receptor of *Periplaneta americana*. *Comput. Biol. Chem.* 32, 185–190.
- Hunter III, J.S., Baggott, D., Everett, W.R., Fourie, J.J., Cramer, L.G., Yoon, S.S., Collidor, N., Mallouk, Y., Lee, L., Blair, J., Prullage, J.B., 2011. Efficacy of a novel combination of fipronil (S)-methoprene and amitraz for treatment and control of brown dog ticks (*Rhipicephalus sanguineus*) on dogs. *Vet. Parasitol.* 179 (2011), 330–334.
- Liu, M.Y., Plapp Jr., F.W., 1992. Mechanisms of formamidine synergism of pyrethroids. *Pestic. Biochem. Physiol.* 43, 134–140.
- Nathanson, J.A., 1985. Phenyliminoimidazolidines. Characterization of a class of potent agonists of octopamine-sensitive adenylate cyclase and their use in understanding the pharmacology of octopamine receptors. *Mol. Pharmacol.* 28, 254–268.
- Orr, G.L., Orr, N., Cornfield, L., Gole, J.W.D., Downer, R.G.H., 1990. Interaction of formamidine pesticides with insect neural octopamine receptors: effects of radioligand binding and cyclic AMP production. *Pestic. Sci.* 30, 285–294.
- Prullage, J.B., Cawthorne, W.G., Le Hir de Fallois, L.P., Timmons, P.R., 2011. Synergy between fipronil and amitraz in a *Rhipicephalus sanguineus* tick residual contact test. *Exp. Appl. Acarol.* (published online 18 January 2011).

- Raymond, V., Sattelle, D.B., 2002. Novel animal-health drug targets from ligand gated chloride channels. *Nat. Rev. Drug Discov.* 1, 427–436.
- Roeder, T., 1995. Pharmacology of the octopamine receptor from locust central nervous tissue (OAR3). *Br. J. Pharmacol.* 114, 210–216.
- Roeder, T., 1999. Octopamine in invertebrates. *Prog. Neurobiol.* 59, 533–561.
- Rugg, D., Hair, J.A., 2007. Dose determination of a novel formulation of metaflumizone plus amitraz for control of cat fleas (*Ctenocephalides felis felis*) and brown dog ticks (*Rhipicephalus sanguineus*) on dogs. *Vet. Parasitol.* 150, 203–208.
- Treacy, M.F., Benedict, J.H., Schmidt, K.M., Anderson, R.M., Wagner, T.L., 1987. Behavior and spatial distribution patterns of tobacco budworm (Lepidoptera: Noctuidae) larvae on chlordimeform-treated cotton plants. *J. Econ. Entomol.* 80, 1149–1151.
- Usmani, K.A., Knowles, C.O., 2001. Toxicity of pyrethroids and effect of synergists to larval and adult *Helicoverpa zea*, *Spodoptera frugiperda*, and *Agrotis ipsilon* (Lepidoptera: Noctuidae). *J. Econ. Entomol.* 94, 868–873.
- Zhang, S.J., Jackson, M.B., 1993. GABA-activated chloride channels in secretory nerve endings. *Science* 259, 531–534.
- Zhao, X., Yeh, J.Z., Salgado, V.L., Narahashi, T., 2005. Sulfone metabolite of fipronil blocks gamma-aminobutyric acid- and glutamate-activated chloride channels in mammalian and insect neurons. *J. Pharmacol. Exp. Ther.* 314, 363–373.
- Zheng, Y., Priest, B., Cully, D.F., Ludmerer, S.W., 2003. RdlDv, a novel GABA-gated chloride channel gene from the American dog tick *Dermacentor variabilis*. *Insect Biochem. Mol. Biol.* 33, 595–599.