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Susceptibility of *Rhipicephalus (Boophilus) microplus* to fluazuron (2.5 mg/kg) and a combination of novaluron (2.0 mg/kg) + eprinomectin (0.36 mg/kg) in field studies in Brazil

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ABSTRACT

The present study aimed to determine the susceptibility of 32 *R. (B.) microplus* populations from Southeast, Midwest and South regions of Brazil, to fluazuron (2.5 mg/kg), administered topically (pour-on). Additionally, five populations (Southeast and Midwest regions) of the southern cattle tick were evaluated using *in vivo* field studies, regarding their susceptibility to a new combination of novaluron (2.0 mg/kg) + eprinomectin (0.36 mg/kg), administered subcutaneously, compared with two positive controls (fluazuron 2.5 mg/kg and eprinomectin 0.5 mg/kg), both administered topically (pour-on). Selected bovines were allocated to treatment groups on day 0, and block formation was based on arithmetic means of female ticks (4.5–8.0 mm long) counted on three consecutive days (−3, −2 and −1). To evaluate therapeutic and residual efficacies of these formulations, tick counts (females ranging from 4.5 to 8.0 mm long) were performed on days 3, 7 and 14 post-treatment, continuing on a weekly basis until the end of each experiment. Results obtained throughout this study, utilizing field efficacy trials, allowed us to conclude that four *R. (B.) microplus* populations (including two in the Southeast and two in the Midwest regions) could be diagnosed as resistant, or with low susceptibility, to fluazuron (2.5 mg/kg). Such fact was detected in farms where owners applied products containing this active component on cattle for at least five years, with treatment intervals of 30–55 days during the rainy season. Nonetheless, *in vitro* studies should be performed in order to reinforce *in vivo* results obtained on the present study. Regarding efficacy indexes obtained by the association of eprinomectin and the novel molecule novaluron against *R. (B.) microplus*, none of the trials managed to obtain efficacies superior to 48%. Such results, allied to data obtained by different researchers and previously published in literature, reinforce the perception that maybe these formulations containing novaluron, in the administered dosages and treatment routes, may not be effective tools for controlling *R. (B.) microplus*. However, future studies must be conducted in order to support such hypothesis. Additionally, all five *R. (B.) microplus* populations were diagnosed as resistant, or with low susceptibility, to eprinomectin (0.5 mg/kg) as well. Even though fluazuron, administered topically (pour on), is still an excellent active principle to be used against *R. (B.) microplus*, resistance management strategies should be quickly implemented in order to keep selection pressure in Brazil at a minimum level for this compound.

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1. Introduction

Rhipicephalus (*Boophilus*) *microplus* is considered one of the most important ectoparasites in cattle industry (Cruz et al., 2014). It has a wide geographical distribution, comprehending tropical and subtropical regions situated between latitude parallels 32° north and 35° south, comprising countries of Latin America, Africa, Asia and Oceania (Wharton, 1974). It is estimated that, in Brazil, losses caused by this tick are close to 3.24 billion dollars per year (Grisi et al., 2014).

Amongst the main tools used for controlling such ectoparasite lately, synthetic chemical compounds stand out, though it is noteworthy that control of *R. (B.) microplus* based on the use of chemical acaricides is mostly done in an indiscriminate manner, without previous knowledge of biological and ecological aspects related to life cycles of ticks (Cruz et al., 2015a). Because of this, reports of tick resistance against several active components are increasing (Castro-Janer et al., 2011; Lopes et al., 2014; Reck et al., 2014; Correa et al., 2015).

For several years, *R. (B.) microplus* control using chemical compounds was done mainly with macrocyclic lactones. However, the rising number of tick populations resistant to this acaricide family (Cruz et al., 2015a) caused the use of such compounds to diminish and, consequently, treatments of animals with different components, such as the benzoylphenyl ureas, to increase (Cruz et al., 2015b; Gomes et al., 2015), which motivated even further the conduction of the present study.

Benzoylphenyl ureas, selective acaricides called insect growth regulators (IGR), act by inhibiting chitin synthesis, making it impossible for larvae and nymphs of ticks to promote ecdysis. Consequently, parasites lose hemolymph and death occurs by dehydration. They show high specificity, low toxicity for mammals and efficacy in low concentrations with a long period of action against adult *R. (B.) microplus* (Retnakaram and Whight, 1987; Graf, 1993; Bull et al., 1996). Within that group stands out fluazuron, first commercialized in Brazil in 1994, which assumes an important role for the control of the aforementioned ectoparasite (Santos et al., 2010). Another IGR belonging the benzoylphenyl urea group is novaluron (1-[3-chloro-4-(1, 1,2-trifuloro-2-trifluoromethoxy-ethoxy) phenyl]-3-(2, 6-difluorobenzoyl) urea). This active principle has very low toxicity to mammals, birds and earthworms (lshaaya and Horowitz, 1998; Barazani, 2001; Ishaaya and Horowitz, 2002), and is widely used in agriculture (Kostyukovsky and Trostanetsky, 2006; Beuzelin et al., 2010). In 2015, a new commercial formulation containing novaluron + eprinomectin became available in Brazilian market, being indicated for treatments against southern cattle ticks, *Rhipicephalus (Boophilus) microplus* (Sindan, 2015).

Considering that fluazuron is the most used active principle against *R. (B.) microplus* in some regions of the world, and there are new active components belong to the same chemical group becoming available commercially to control the southern cattle tick, such as novaluron, information about *R. (B.) microplus* susceptibility to these compounds is essential in order to create strategies for developing a progressive and more rational chemical control strategy against this ectoparasite in cattle (Lopes et al., 2014).

Therefore, the present study aimed to determine the susceptibility of 32 *R. (B.) microplus* populations, from the Southeast, Midwest and Southern regions of Brazil, to fluazuron (2.5 mg/kg), administered topically (pour on). Additionally, five populations (Southeast and Midwest regions) of the southern cattle tick were evaluated regarding their susceptibility to the new molecule of novaluron (2.0 mg/kg) associated to eprinomectin (0.36 mg/kg), administered subcutaneously, compared with two positive controls (fluazuron 2.5 mg/kg and eprinomectin 0.5 mg/kg), both administered topically (pour-on), by means of *in vivo* field studies.

2. Materials and methods

2.1. Study locations, division of groups, dose determination procedures and tick counts

Between February 2012 and March 2016, experiments were conducted in different rural properties of Brazil. All studies were performed between the months of October and May, in order to ensure that the challenge of animals with *R. (B.) microplus* would be in accordance with population dynamic data of this ectoparasite in the regions where all 32 studies were conducted. Such data, obtained by several different authors, demonstrate that this tick species present an average of thre to four tick generations during this period of the year, considered to be three rainy season in Brazil (Kasai et al., 2000; Martins et al., 2002; Pereira et al., 2008; Gomes et al., 2016). A total of 32 *R. (B.) microplus* populations were evaluated regarding their susceptibility to fluazuron, administered topically (pour on) at a dose of 2.5 mg/kg. Five populations were tested against a novel combination of novaluron (2.0 mg/kg) + eprinomectin (0.36 mg/kg), as well as a formulation of eprinomectin (0.50 mg/kg), administered by subcutaneous and pour on routes, respectively. For fluazuron, thirteen tick populations were selected on the state of São Paulo and ten came from the state of Minas Gerais, both located on the Southeast region of Brazil. Three tick strains originated at the state of Mato Grosso do Sul and four from Goiás, states from the Midwest region of Brazil. The final two tick populations were from the state of Paraná, South region of Brazil. Regarding novaluron + eprinomectin and eprinomectin, three tick populations from the state of São Paulo and two from Goiás were evaluated regarding the efficacy of these formulations. The chemical compounds used at each property, as well as the history of fluazuron usage on each herd since the beginning of its administration are described in Table 1.

For experiments 1–27, 20 mixed breed bovines (*Bos taurus* x *Bos indicus*) naturally infested by *R. (B.) microplus*, with ages ranging from 8 to 18 months, were utilized. In each of these experiments, animals were distributed into two groups of ten animals each. Treatment 01 received saline solution, while treatment 02 received fluazuron topically (pour on) at a dosage of 2.5 mg/kg. In experiments 28–32, 40 mixed breed animals (*Bos taurus* x *Bos indicus*) naturally infested by *R. (B.) microplus*, with ages ranging from 8 to 18 months, were selected. In this case, bovines were divided into four groups of ten animals each. Treatment 01 received saline solution; treatment 02 received 2.0 mg/kg novaluron + 0.36 mg/kg eprinomectin subcutaneously; animals belonging to treatment 03 were treated with 2.5 mg/kg fluazuron topically (pour on); and treatment 04 received 0.5 mg/kg of eprinomectin topically (pour on). Due to the fact that novaluron is a novel active component, recently marketed in Brazil against southern cattle ticks, fluazuron (2.5 mg/kg) and eprinomectin (0.5 mg/kg) were used as reference products. Counting all studies, the total number of experimental animals was 740. For each of the trials, the same category of animals was selected, so that body weight of animals did not differ statistically between treated and control groups. Only clinically healthy bovines with a good nutritional condition were selected for all experiments. Cattle chosen for all studies had not been medicated for a period of at least 90 days before the beginning of each experiment. All animals were maintained on a grazing regimen, following the one already in use on each rural property.

Bovines were allocated to experimental groups on day 0, being randomly designated to treatments according to a masked complete block design. Block formation was based on arithmetic means

Table 1
Usage history of fluazruon in the properties where the studies were performed.

Study	Region of Brazil	Farm city – State	Month and year of begining the study	Product[1] used	Route	How long uses the product? (years)	Frequence of use of fluazuron
1	Southeast	Pimenta – MG	January 2013	fluazuron	pour-on	two	at least two times per year
2	Southeast	Campo Florido – MG	Febuary, 2012	fluazuron	pour-on	three	at least two times per year
3	Southeast	Passos – MG	November of 2014	fluazuron	pour-on	two	at least two times per year
4	Southeast	Poços de Caldas – MG	January 2015	fluazuron	pour-on	three	the owner could not say
5	Southeast	Formiga – MG	October 2015	fluazuron	pour-on	eigth	at least two times per year
6	Southeast	São Sebastião do Paraíso – MG	Febuary, 2012	fluazuron	pour-on	four	at least tree times per year
7	Southeast	Arcos – MG	December 2014	fluazuron	pour-on	five	each 40–45 days of interval
8	Southeast	Divinópolis- MG	January 2016	fluazuron	pour-on	four	at least three times per year
9	Southeast	Pains- MG	Febuary, 2014	fluazuron	pour-on	five	at least three times per year
10	Southeast	Caldas – MG	March 2013	fluazuron	pour-on	five	at least four times per year
11	Southeast	Franca – SP	November 2014	fluazuron	pour-on	never used	not applied
12	Southeast	Casa Branca – SP	Febuary, 2013	fluazuron	pour-on	one	two times of use
13	Southeast	Aguas da Prata – SP	December 2013	fluazuron	pour-on	one	one time
14	Southeast	Itirapuã – SP	March 2014	fluazuron	pour-on	two	three to six times since the begin
15	Southeast	Gastão Vidgal – SP	January 2013	fluazuron	pour-on	six	each 30–40 days of interval
16	Southeast	São João da Boa Vista – SP	December 2015	fluazuron	pour-on	seven	three to six times since the begin
17	Southeast	São José do Rio Pardo – SP	January 2015	fluazuron	pour-on	five	at least three times per year
18	Southeast	Santo Antônio de Posse- SP	Febuary, 2014	fluazuron	pour-on	five	at least five times per year
19	Southeast	Vargem Grande do Sul- SP	March 2013	fluazuron	pour-on	two	three to six times since the begin
20	Southeast	São Sebastião da Grama- SP	October, 204	fluazuron	pour-on	five	the owner could not say
21	Midwest	Bandeirantes – MS	December 2012	fluazuron	pour-on	more than five	at least four times per year
22	Midwest	Terenos – MS	December 2012	fluazuron	pour-on	more than five	at least four times per year
23	Midwest	Jaguari – MS	Febuary, 2014	fluazuron	pour-on	more than five	at least four times per year
24	Midwest	Caiapônia – GO	March 2015	fluazuron	pour-on	three	the owner could not say
25	Midwest	Jataí – GO	January 2015	fluazuron	pour-on	never used	not applied
26	South	Icaraíma – PR	January 2015	fluazuron	pour-on	four	the owner could not say
27	South	Umuarama – PR	December 2014	fluazuron	pour-on	three	at least four times per year
28	Southeast	Espirito Santo do Pinhal – SP	November of 2015	fluazuron, eprinimectin and noval-uron + eprinomectin	subcutaneous	five	three to eight times since the begin
29	Southeast	São João da Boa Vista – SP	November of 2015	fluazuron, eprinimectin and noval-uron + eprinomectin	subcutaneous	five	at least four times per year
30	Southeast	Tambaú – SP	January 2016	fluazuron, eprinimectin and noval-uron + eprinomectin	subcutaneous	three	the owner could not say
31	Midwest	Jataí – GO	Febuary, 2016	fluazuron, eprinimectin and noval-uron + eprinomectin	subcutaneous	three months	two times of use
32	Midwest	Acreúna – GO	March 2016	fluazuron, eprinimectin and noval-uron + eprinomectin	subcutaneous	four	at least four times per year

[2] Commercial formulation purchased in the local market.

of female ticks (measuring 4.5 to long 8.0 mm) counted on three consecutive days (−3, −2 and −1), as recommended by Wharton and Utech (1970). In each experiment, animals were distributed into ten blocks containing two or four bovines, and these were randomly placed in one of the treatment groups inside each block. The number of bovines per group used in all experiments (10 animals per group) was determined in accordance with recommendations described by the Brazilian Ministry of Agriculture, Livestock and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento – MAPA), Ordinance number 48 (Brazil, 1997).

Acaricide compounds used for treatment of experimental animals were commercial products available in the Brazilian veterinary market that contained 2.5 mg/kg fluazuron, administered at pour-on route (Acatak® – Novartis Animal Health); 2.0 mg/kg novaluron + 0.36 mg/kg eprinomectin, administered subcutaneously (Novatak Gold® – Clarion Animal Health); and 0.5 mg/kg eprinomectin (Eprinex® – Merial Animal Health). All products were stored and used according to label specifications. In each experiment, cattle were individually weighed one day prior to treatment (day −1) in order to allow calculations of the correct dosage. It is important to reinforce that scales were previously tested and verified for accuracy. Each treatment was performed individually in each animal, and the administration of products was always performed by a veterinarian.

To ensure dosing techniques were conducted using similar standards, volumes administered in each experiment were calculated using the measured individual weight of each animal before treatment and, if necessary, were rounded down in 0.1 mL. For example: an animal of 176 kg, which would receive 17.6 mL using a pour-on product, therefore received 17.5 mL, and an animal of the same weight, which would receive 3.52 mL using an injectable product, therefore received 3.5 mL. All bovines were appropriately restrained in a chute during treatment. It is important to report that cattle treated with pour-on formulations did not suffer interference of rain in the first 72 h post-treatment, with the exception of studies 1 (Pimenta city), 2 (Campo Florido city), 8 (Divinópolis city), 13 (Águas da Prata city) and 25 (Jataí city), where presence of rain (approximately 15 mm^3) was registered in the first 24 h after treatment of animals.

Additional effects of fluazuron (2.5 mg/kg) and the novaluron (2.0 mg/kg) + eprinomectin (0.36 mg/kg) association on the reproductive parameters of field populations of *Rhipicephalus (Boophilus) microplus* were not evaluated on the present study, since the methodology recommended for such analysis is adapted for Stall Test studies, using experimentally infested bovines.

According to available works in literature, there is only one report of *R. (B.) microplus* resistant to fluazuron (Reck et al., 2014) so far. In Brazil, the number of products containing benzoylphenyl ureas, such as fluazuron and novaluron, the last molecule to become commercially available in the country, are widely increasing. Such factor further motivated the conduction of all 32 experiments using these actives compounds.

During all experimental periods of each trial, in all selected farms, untreated control groups and groups treated with the injectable formulation (novaluron + eprinomectin) were maintained in the same paddock, formed from natural pastures of each location. In specific instances when pour-on formulations were applied (fluazuron and eprinomectin), animals from different groups were kept in separate paddocks during the first seven days after treatment, allowing the products to be totally absorbed, consequently preventing any possibility of contaminating untreated control groups. Drinkable water and mineral supplementation were provided for all experimental cattle *ad libitum* during all of the experiments, following previously established handling procedures at each property.

To evaluate therapeutic and residual efficacy indexes of each formulation, tick counts (females between 4.5 and 8.0 mm long) were performed on days 3, 7 and 14 post-treatment, continuing every 7 days thereafter until the end of each trial, according to the technique described by Wharton and Utech (1970). To ensure that tick counting procedures were conducted consistently between time points and study locations, counts in each experiment were performed at the same time of day and by the same person (a veterinarian), on all experimental dates before and after treatment. Moreover, each person carried a metal card containing holes measuring 4.5 and 8.0 mm long (scale tested), to ensure that only ticks from the same size range were counted. Animals treated with injectable solutions and untreated controls were counted first, followed by groups which received pour-on topical treatments, in order to prevent any possible contamination. In all studies, disposable gloves were used and changed between tick counts on each animal. All experiments were blinded; thus, completely reliable data were obtained.

Before the implementation of the present study, all procedures using animals were approved by the Ethical Committee for Animal Welfare of IPESA, under protocol number 121/12, being, therefore, considered in compliance with the Ethical Principles in Animal Research, adopted by the College of Animal Experimentation (COBEA).

2.2. Data analysis

Raw counts of partially engorged female ticks on experimental cattle were log transformed, using the equation ln (x + 1), prior to statistical analysis, and underwent the UNIVARIATE procedure. Subsequently, all data were analyzed by the GLM procedure using the REPEATED command, to test sphericity and orthogonality of data, which determined that the sphericity condition of the matrix should not be rejected (SAS, 1996). Differences between treatments were evaluated at a significance level of 5% ($P \leq 0.05$).

Efficacy percentages obtained against *R. (B.) microplus* were calculated using arithmetic means, according to a formula proposed by Roulston et al. (1968) and adopted by the Brazilian Ministry of Agriculture, Livestock and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento – MAPA), Ordinance number 48 (Brazil, 1997), as described below:

$$\text{Efficacy percentage} = \left[1 - \frac{TaxCb}{TbxCa}\right] x100$$

In this equation, **Ta** represents the mean number of partially engorged female ticks counted on treated animals after medication; **Tb** is the mean number of partially engorged female ticks counted on treated animals during the three days that preceded treatment; **Ca** is the mean number of partially engorged female ticks counted on the control group after the experiment was initiated; and **Cb** is the mean number of partially engorged females counted on untreated animals (control) during the three days that preceded treatment.

2.3. Criteria for diagnosis of susceptibility or resistant/low susceptibility of a tick population to fluazuron, novaluron and eprinomectin

According to EMEA (2004) and Holdsworth et al. (2006), with some exceptions, the control of a tick population by a specific formulation is considered unsatisfactory when efficacy values of such compound are inferior to 90%.

As described by Holdsworth et al. (2006), for testing products with a slower onset of action, such as benzoylphenyl ureas, selective acaricides (IGR), draft product labels must indicate the period of time required to kill a designated percentage of ticks on treated ruminants, based on valid scientific data. Under such criteria, and considering previous studies which demonstrated that 2.5 mg/kg fluazuron takes about 14 days to present its satisfactory effects against this cattle tick (Cruz et al., 2014; Gomes et al., 2015), *R. (B.) microplus* populations subjected to fluazuron and novaluron were considered susceptible when these compounds showed an efficacy greater than 90% (EMEA, 2004; Holdsworth et al., 2006) after the 14th day post treatment.

For eprinomectin, the Brazilian Ministry of Agriculture, Livestock and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento – MAPA), Ordinance number 48 (Brazil, 1997),

states the importance of considering mean efficacies of a formulation between days 7 and 14 post-treatment. A mean efficacy superior to 90% between these dates was the criteria adopted to designate *R. (B.) microplus* as susceptible to eprinomectin, coupled with the fact that peak plasma levels of this same active formulation (1.0 and 1.5 mg/kg) in treated cattle occurs between 2 and 4 days after administration (Lifschitz et al., 2016).

Therefore, populations of *R. (B.) microplus* where the efficacy indexes of different compounds were inferior 90%, on experimental dates cited above, were classified as resistant, or with low susceptibility, to such compounds.

Studies indicate that arithmetic means should be used to calculate the efficacy indexes of a formulation against target parasites (Dobson et al., 2009; Vercruysse et al., 2011; Cruz et al., 2015b).

3. Results

No signs of abnormalities or systemic intoxication were observed in experimental animals before or after administration of tested formulations in all 32 studies.

3.1. Fluazuron 2.5 mg/kg (Minas gerais state)

Efficacy values obtained by fluazuron on studies conducted at the state of Minas Gerais (Southeast region of Brazil) are described in Table 2. Based on such results, it is possible to verify that, amongst all ten evaluated *R. (B.) microplus* populations, only one, located on the city of Arcos, was diagnosed as resistant, or with low susceptibility, to fluazuron, administered topically (pour-on) at a dosage of 2.5 mg/kg. At this trial, the aforementioned formulation reached a maximum efficacy index of 87.1% on the 21st DPT (Table 2). In three other experiments conducted on the same state, fluazuron reached maximum efficacy values (100%) against *R. (B.) microplus* between the 14th and 21st DPT. When analyzing the residual period, in days, during which this formulation maintained efficacy levels superior or equal to 90% after the 14th DPT, it was possible to verify that residual efficacy lasted 21 days in the city of Caldas and up to 42 days on the cities of Pimenta, Campo Florido and Passos (Table 2).

3.2. Fluazuron 2.5 mg/kg (São paulo state)

Considering the criteria, previously established on the present study, for diagnosing susceptibility of *R. (B.) microplus* populations to fluazuron (2.5 mg/kg), it was possible to diagnose one tick population as resistant, or at least with low susceptibility, to such formulation on the state of São Paulo (Southeast Brazil), at Gastão Vidigal city, in a similar way to what happened in Minas Gerais. In this particular experiment, the highest efficacy index obtained by fluazuron was 83.2%, 5 days after treatment of bovines. Maximum efficacy (100%) against *R. (B.) microplus* was obtained by this formulation in two other trials on the same state, at the cities of Franca and Vargem Grande do Sul. Residual acaricide periods (efficacies superior or equal to 90% after the 14th DPT) were observed by fluazuron for up to 14 and 63 days after treatment on the cities of Santo Antonio de Posse and Franca, respectively (Table 3).

3.3. Fluazuron 2.5 mg/kg (Mato grosso do sul and goiás states)

On the states of Mato Grosso do Sul and Goiás (Center-West region of Brazil), between all seven *R. (B.) microplus* populations evaluated, two could be diagnosed as resistant, or with low susceptibility, to fluazuron (2.5 mg/kg), both on the state of Mato Grosso do Sul. In one of these populations, maximum efficacy obtained by such formulation was 40.8% on the 14th DPT (Table 4). Residual efficacies against *R. (B.) microplus* were presented by fluazuron (indexes $\geq$ 90% after the 14th DPT), with values of 28 and 42 days post treatment, on the cities of Icaraíma and Umuarama, respectively (Table 4).

3.4. Fluazuron 2.5 mg/kg and Novaluron 2.0 mg/kg + Eprinomectin 0.36 mg/kg (São Paulo and Goiás States)

Regarding efficacy values obtained on all five trials, that evaluated indexes obtained by the novaluron + eprinomectin combination, in comparison to fluazuron and eprinomectin, it was possible to verify that all analyzed tick populations were susceptible to fluazuron (2.5 mg/kg). On the other hand, when looking at results obtained by the novel combination of novaluron associated to eprinomectin, it can be observed that, in all tested *R. (B.) microplus* populations, efficacy indexes of such combination were always inferior to 48.0%. Similar results were demonstrated by the pour-on eprinomectin formulation, since such drug, administered alone, reached maximum efficacy of 57.8% (14th DPT) in the city of Espírito Santo do Pinhal (Table 5). Based on obtained data, and considering criteria previously established for diagnosing susceptibility of *R. (B.) microplus* populations to a benzoylphenyl urea and eprinomectin, all tested tick populations were diagnosed as resistant, or with low susceptibly, to the novaluron (2.0 mg/kg) + eprinomectin (0.36 mg/kg) combination and to eprinomectin (0.5 mg/kg), administered through subcutaneous and pour on routes, respectively (Table 5).

4. Discussion

Regarding fluazuron effects against *Rhipicephalus (Boophilus) microplus,* Santos et al. (2010), using artificial infestation trials known as stall tests, detected a residual acaricide effect (efficacy $\geq$ 90%) of 95 and 92 days post treatment for 2.5 mg/kg fluazuron and 3.0 mg/kg fluazuron + 0.5 mg/kg abamectin, respectively. In another southern cattle tick population, Gomes et al. (2015), in two studies of natural and artificial (stall test) infestations, detected efficacies superior or equal to 90%, that lasted for 49 and 77 days after treatment of animals, respectively. Using field trials on the present study, residual acaricide effects of fluazuron 2.5 mg/kg ranged between 14 and 63 days, when these formulations presented efficacy values $\geq$ 90%. Bull et al. (1996), in an article on *R. (B.) microplus*, observed that practically no adult tick was recorded between 3 and 12 weeks after treatment of cattle with fluazuron. However, it is important to mention that *R. (B.) microplus* populations from Australia were recently reclassified as *Rhipicephalus (Boophilus) australis* (Estrada-Peña et al., 2012; Ali et al., 2016), which means that, probably, the tick species involved in the study of Bull et al. (1996) is actually *R. (B.) australis* instead of *R. (B.) microplus*.

In the present study, the fact that efficacy values of fluazuron took approximately 15 days to overcome 90% levels can be justified by action mechanisms of this particular acaricide class, since such compounds are inhibitors of chitin synthesis. Consequently, it takes more time until larvae and nymphs are affected by this active principle (Retnakaram and Whight, 1987; Holdsworth et al., 2006).

The first report of a *R. (B.) microplus* population resistant to fluazuron was recently published by Brazilian researchers (Reck et al., 2014). This specific strain originated from the Rio Grande do Sul State, South region of Brazil, and is also the first with documented resistance to all six classes of acaricides used to control cattle tick in this country: organophosphates, formamidines (amitraz), synthetic pyrethroids, macrocyclic lactones, phenylpyrazoles (fipronil) and benzoylphenyl ureas (fluazuron). Based on results obtained by the present study, it is possible to verify that there are also *R. (B.) microplus* populations resistant, or presenting low susceptibility, to

Table 2
Mean counts of female *R. (B.) microplus* (4.5–8.0 mm long) for control and treated cattle groups; results of variance analysis of tick counts and efficacy percentages in experiments performed in Southeast region of Brazil (Minas Gerais State).

Day of study	T01: Control (Saline solution)					T02: Fluazuron (2.5 mg/kg)[2]					Efficacy (%)	Classification of *R. (B.) microplus* strain to fluazuron[3]	Residual efficacy of fluazrunon to *R. (B.) microplus*[4]
	Mean tick count[1]		Range			Mean tick count[1]		Range			Arithmetic mean		
Experiment 1 – Pimenta city													
0	26.8	A	10.0	–	31.7	26.9	A	10.3	–	33.7	–	Susceptible	42
3	28.1	A	10	–	54	26.6	A	14	–	38	14.3		
7	28.6	A	18	–	53	15.1	A	3	–	40	52.2		
14	48.7	A	24	–	106	1.7	B	0	–	6	96.8		
21	29.0	A	18	–	55	0.0	B	0	–	0	100.0		
28	25.5	A	16	–	54	0.0	B	0	–	0	100.0		
35	25.5	A	18	–	36	0.3	B	0	–	2	98.9		
42	21.9	A	16	–	29	1.4	B	0	–	3	94.2		
49	23,2	A	8	–	38	9.7	B	4	–	16	62,1		
Experiment 2 – Campo Florido city													
0	27.7	A	14.0	–	72.3	25.2	A	14.3	–	41.0	–	Susceptible	42
3	26.0	A	11	–	58	17.7	A	3	–	51	25.2		
7	32.3	A	7	–	87	18.7	B	2	–	117	36.4		
14	32.2	A	5	–	91	0.6	B	0	–	3	98.0		
21	25.3	A	6	–	65	0.2	B	0	–	1	99.1		
28	20.2	A	8	–	49	0.6	B	0	–	4	96.7		
35	19.1	A	8	–	36	0.6	B	0	–	2	96.5		
42	18.5	A	7	–	33	1.5	B	0	–	11	91.1		
49	24.8	A	6	–	57	5.7	B	0	–	26	74.7		
56	18.1	A	9	–	42	8.7	B	0	–	38	47.2		
Experiment 3 – Passos city													
0	40.1	A	23.7	–	40.0	40.4	A	20.3	–	41.3	–	Susceptible	42
3	40.7	A	26	–	55	42.7	A	15	–	61	0.0		
7	42.3	A	28	–	68	37.6	A	10	–	45	11.9		
14	39.8	A	26	–	59	2.5	B	0	–	8	93.8		
21	37.3	A	26	–	51	0.0	B	0	–	0	100.0		
28	36.8	A	23	–	53	0.8	B	0	–	1	97.8		
35	31.8	A	18	–	45	1.7	B	0	–	3	94.8		
42	31.5	A	21	–	43	3.0	B	1	–	5	90.6		
49	31.3	A	17	–	41	8.5	B	7	–	21	73.1		
Experiment 4 – Poços de Caldas city													
0	21.9	A	9.7	–	45.7	21.4	A	13.7	–	34.0	–	Susceptible	28
3	32.6	A	8	–	59	25.6	B	4	–	54	19.5		
7	19.1	A	9	–	43	14.4	B	1	–	54	22.7		
14	18.2	A	8	–	34	5.6	B	0	–	16	68.5		
21	21.1	A	7	–	82	1.4	B	0	–	6	93.2		
28	21.7	A	7	–	84	0.2	A	0	–	1	99.1		
35	25.0	A	5	–	89	6.1	B	3	–	12	75.0		
Experiment 5 –Formiga city													
0	26.8	A	12.0	–	60.0	26.5	A	12.3	–	54,0	–	Susceptible	28
3	30.6	A	15	–	55	26.4	A	12	–	51	12.7		
7	31.6	A	13	–	58	20.1	A	2	–	34	35.6		
14	28.3	A	16	–	42	1.7	B	0	–	6	93.9		
21	35.4	A	10	–	68	0.1	B	0	–	1	99.7		
28	41.4	A	11	–	79	0.7	B	0	–	5	98.3		
35	43.4	A	13	–	81	12.3	B	0	–	78	71.4		
42	47.9	A	9	–	87	15.5	B	3	–	114	67.4		
Experiment 6 – São Sebastião do Paraíso city													
0	46.1	A	23.8	–	78.7	46.3	A	25.7	–	79.8	–	Susceptible	35
3	47.3	A	26	–	76	25.2	A	13	–	37	47.1		
7	44.5	A	28	–	81	16.0	A	12	–	23	64.2		
14	46.3	A	26	–	98	1.0	B	0	–	3	97.9		
21	42.8	A	21	–	90	0.8	B	0	–	1	99.6		
28	42.5	A	23	–	87	1.0	B	0	–	4	97.7		
35	37.8	A	18	–	81	1.33	B	0	–	5	96.5		
42	38.7	A	21	–	86	4.7	B	2	–	12	88.0		
49	31.3	A	17	–	41	11.0	B	3	–	21	65.1		
Experiment 7 – Arcos city													
0	50.2	A	17.3	–	138.0	59.1	A	18.1	–	138.7	–	Resistant or with low susceptibility	Not apllied
3	56.7	A	18	–	108	50.0	A	12	–	117	25.1		
7	59.0	A	14	–	137	31.8	B	3	–	101	54.2		
14	88.1	A	22	–	227	24.6	B	2	–	72	76.3		

Table 2 (*Continued*)

Day of study	T01: Control (Saline solution)					T02: Fluazuron (2.5 mg/kg)[2]					Efficacy (%)	Classification of *R. (B.) microplus* strain to fluazuron[3]	Residual efficacy of fluazrunon to *R. (B.) microplus*[4]
	Mean tick count[1]		Range			Mean tick count[1]		Range			Arithmetic mean		
Experiment 1 – Pimenta city													
21	83.8	A	28	–	180	12.7	B	1	–	78	87.1		
28	87.7	A	33	–	168	15.9	B	1	–	38	84.6		
35	83.4	A	38	–	143	23.6	B	2	–	69	76.0		
42	79.0	A	41	–	135	13.0	B	1	–	36	86.0		
49	43.0	A	13	–	86	13.7	B	0	–	31	73.0		
Experiment 8 – Divinópolis city													
0	46.8	A	32.1	–	67.7	46.6	A	32.3	–	67.3	–	Susceptible	28
3	39.8	A	30	–	69	39.8	A	31	–	43	0.0		
7	43.2	A	27	–	76	39.7	A	21	–	36	7.6		
14	44.5	A	25	–	79	0.5	B	0	–	4	98.9		
21	43.4	A	23	–	81	0.0	B	0	–	0	100.0		
28	46.7	A	22	–	81	1.3	B	0	–	7	97.2		
35	44.5	A	26	–	86	4.5	B	0	–	21	89.8		
42	48.7	A	32	–	84	15.6	B	1	–	45	67.8		
Experiment 9 – Pains city													
0	37.3	A	18.7	–	78.3	37.7	A	18.3	–	77.7	–	Susceptible	35
3	32.5	A	21	–	77	32.0	A	14	–	71	2.6		
7	38.7	A	19	–	79	31.1	A	15	–	54	20.5		
14	39.8	A	21	–	77	2.1	B	0	–	7	94.8		
21	37.6	A	17	–	67	1.4	B	0	–	6	96.3		
28	41.5	A	18	–	69	1.7	B	0	–	9	95.9		
35	42.5	A	18	–	74	3.5	B	0	–	11	91.9		
42	42.9	A	19	–	78	13.5	B	0	–	78	68.9		
49	40.5	A	18	–	74	21.6	B	2	–	79	47.2		
Experiment 10 – Caldas city													
0	53.6	A	21.6	–	71.3	53.3	A	21.3	–	71.3	–	Susceptible	21
3	56.7	A	20	–	78	56.7	A	19	–	78	0.0		
7	59.8	A	19	–	76	51.0	A	10	–	56	14.2		
14	61.7	A	23	–	74	5.6	B	3	–	9	90.9		
21	63.5	A	21	–	79	5.8	B	1	–	7	90.8		
28	65.5	A	23	–	81	10.5	B	3	–	31	83.9		
35	67.8	A	22	–	83	23.6	B	8	–	45	65.0		

[1] Means values followed by the same letter on the same line do not differ significantly at a 95% reliability level.
[2] Commercial formulation purchased in the local market.
[3] Rating susceptible performed based on the efficacy (>90%) using the arithmetic mean.
[3] Rating resistant performed based on the efficacy (≤90%) using the arithmetic mean.
[4] The last day of the study that the chemical compund show efficacy ≥90%.

fluazuron (2.5 mg/kg) on the states of Minas Gerais, São Paulo and Mato Grosso do Sul (Southeast and Center-West regions of Brazil).

The therapeutic efficacy of a formulation can be defined as the effect a certain product causes against ticks present on the entire body surface of an animal on treatment day. Residual efficacy, on the other hand, can be defined as the effects presented by a formulation against new infestations of the treated animal with more ticks. At this study, even though rain was present in some experiments (trial #1 in Pimenta city, trial #8 in Divinópolis city, trial #13 in Águas da Prata city and trial #25 in Jataí city), 24 h after treatment of animals with pour on fluazuron, such fact did not interfere on therapeutic efficacies of the aforementioned product since, even in these trials where rain was present, fluazuron reached anti-*R. (B.) microplus* efficacy indexes between 98.7% and 100% on the 21st DPT. According to Silva et al. (2015), rainfall, or even incidence of sunlight and dew over animals' coats, can eventually interfere on residual efficacy indexes of a topically administered formulation. Results obtained on studies conducted by such researchers reinforce the aforementioned inference. Silva et al. (2015), in studies with *R. (B.) microplus*, using artificially and naturally infested animals kept in pasture conditions, report that animals treated with pour on formulations and maintained in the stall test did not suffer interference of rain or direct sunlight. Unlike those, animals treated with the same compound and maintained in field conditions, in turn, suffered interference in the residual action period of a compound. Therefore, differences in residual efficacy periods (during which the product maintained efficacies ≥ 90% after the 14th DPT) of fluazuron, observed on artificial infestation studies (stall tests) by Santos et al. (2010) and Gomes et al. (2015), in comparison to those observed on the present study, can be justified by reports described above.

Other relevant aspect, that must be mentioned in this article is that *R. (B.) microplus* populations diagnosed as resistant, or at least less susceptible, against fluazuron (2.5 mg/kg) were the ones present in farms where owners used such formulation on herds for more than five years, with treatment intervals of 30–55 days between them during the rainy season.

Novaluron is also a chitin synthesis inhibitor, that acts by both ingestion and contact, particularly targeting immature stages that actively synthesize chitin. Previous works have shown that novaluron is effective against a variety of insects, including Coleoptera, Homopterta, Hymenoptera, Lepidoptera and Diptera (Malinowski and Pawinska, 1992; Glowacka and Malinowski, 1994; Pluciennik et al., 1999; Barazani, 2001; Ishaaya and Horowitz, 1998, 2002; Cutler et al., 2005; Mascari et al., 2007; Arredondo-Jimenez and Valdez-Delgado, 2006). This active component showed satisfactory efficacy values against insects parasitizing grains and sugarcane (Kostyukovsky and Trostanetsky, 2006; Beuzelin et al., 2010). The

Table 3
Mean counts of female *R. (B.) microplus* (4.5–8.0 mm long) for control and treated cattle groups; results of variance analysis of tick counts and efficacy percentages in experiments performed in Southeast region of Brazil (São Paulo State).

Day of study	T01: Control (Saline solution)					T02: Fluazuron (2.5 mg/kg)[2]					Efficacy (%)	Classification of *R. (B.) microplus* strain to fluazuron[3]	Residual efficacy of fluazrunon to *R. (B.) microplus*4
	Mean tick count[1]		Range			Mean tick count[1]		Range			Arithmetic mean		
Experiment 11- Franca city													
0	52,3	A	23.7	–	93.0	51,9	A	27	–	82	–	Susceptible	63
3	46.5	A	24	–	85	40,7	A	17	–	69	11.8		
7	44.7	A	22	–	76	27,9	B	9	–	59	37.1		
14	32.3	A	18	–	57	5,3	B	0	–	27	91.9		
21	25.7	A	8	–	76	0,1	B	0	–	1	99.6		
28	22.2	A	10	–	34	0,0	B	0	–	0	100.0		
35	24.4	A	12	–	30	0,2	B	0	–	2	99.2		
42	18.6	A	6	–	27	0,8	B	0	–	3	98.2		
49	21.8	A	13	–	32	0,9	B	0	–	8	98.5		
56	37.0	A	21	–	61	4,6	B	0	–	28	94.3		
63	30.3	A	20	–	43	3,3	B	0	–	11	93.4		
70	31.3	A	15	–	57	13,6	B	1	–	38	63.3		
77	31.9	A	17	–	52	19,6	B	9	–	34	35.1		
Experiment 12 – Casa Branca city													
0	40.5	A	26.3	–	55.6	40.6	A	25.7	–	57.6	–	Susceptible	49
3	52.9	A	18	–	101	49.4	A	31	–	82	6.8		
7	64.2	A	31	–	134	50.3	A	31	–	69	21.8		
14	63.5	A	33	–	123	3.5	B	0	–	10	94.5		
21	61.3	A	23	–	100	0.3	B	0	–	2	99.5		
28	51.6	A	16	–	87	0.1	B	0	–	1	99.8		
35	50.6	A	13	–	80	0.2	B	0	–	1	99.6		
42	49.3	A	10	–	75	0.5	B	0	–	1	99.0		
49	46.5	A	12	–	69	2.6	B	0	–	2	94.4		
56	43.3	A	13	–	69	8.2	B	3	–	23	81.1		
63	44.1	A	25	–	59	12.0	B	3	–	25	72.9		
Experiment 13 – Aguas da Prata city													
0	56.1	A	32.7	–	86.7	55.6	A	32,0	–	83.7	–	Susceptible	28
3	63.8	A	32	–	105	54.2	B	31	–	98	14.3		
7	58.9	A	19	–	127	49.8	B	10	–	101	14.7		
14	46.2	A	16	–	135	1.0	B	0	–	4	97.8		
21	45.6	A	12	–	135	0.4	B	0	–	2	99.1		
28	52.0	A	11	–	148	0.8	B	0	–	3	98.4		
35	27.5	A	13	–	59	4.1	B	0	–	20	85.0		
42	28.7	A	18	–	51	10.3	B	1	–	26	63.8		
49	28.1	A	16	–	52	12.3	B	3	–	28	55.8		
Experiment 14 – Itirapuã city													
0	36.9	A	20.3	–	154.7	36.9	A	19.7	–	158.7	–	Susceptible	35
3	38.4	A	18	–	169	30.0	A	14	–	127	21.9		
7	43.4	A	14	–	188	26.7	B	16	–	103	38.5		
14	32.8	A	10	–	150	4.4	B	0	–	14	86.6		
21	28.8	A	8	–	127	1.7	B	0	–	11	94.1		
28	28.1	A	9	–	121	0.9	B	0	–	7	96.8		
35	29.9	A	7	–	97	3.0	B	0	–	17	90.0		
42	30.6	A	8	–	102	4.7	B	0	–	21	84.7		
49	35.2	A	12	–	103	10.4	B	3	–	30	70.5		
56	29.0	A	14	–	87	12.3	B	6	–	29	57.6		
Experiment 15 – Gastão Vidgal city													
0	68.1	A	22.0	–	146.7	68.2	A	20.67	–	173.7	–	Resistant or with low susceptibility	Not applied
3	67.5	A	41	–	141	51.9	A	17	–	103	23.2		
7	67.1	A	48	–	134	30.8	B	15	–	60	54.1		
14	58.6	A	44	–	76	12.3	B	8	–	32	79.0		
21	48.6	A	33	–	74	9.9	B	2	–	18	79.7		
28	42.7	A	29	–	61	7.5	B	0	–	23	82.6		
35	40.4	A	18	–	67	6.8	B	3	–	25	83.2		
42	47.2	A	21	–	72	15.6	B	6	–	32	67.0		
Experiment 16 – São João da Boa Vista city													
0	33.1	A	13.7	–	64.7	34.3	A	12.3	–	71.7	–	Susceptible	42
3	28.1	A	12	–	65	24.9	A	9	–	48	14.5		
7	28.0	A	17	–	51	15.5	B	3	–	58	46.6		
14	33.0	A	15	–	57	2.3	B	0	–	5	93.3		
21	30.0	A	10	–	51	0.1	A	0	–	1	99.7		
28	30.4	A	8	–	65	1.3	B	0	–	5	95.9		
35	34.5	A	7	–	73	1.9	B	0	–	6	94.7		

Table 3 (*Continued*)

Experiment 11- Franca city													
Day of study	T01: Control (Saline solution)					T02: Fluazuron (2.5 mg/kg)[2]					Efficacy (%)	Classification of *R. (B.) microplus* strain to fluazuron[3]	Residual efficacy of fluazrunon to *R. (B.) microplus*4
	Mean tick count[1]		Range			Mean tick count[1]		Range			Arithmetic mean		
42	41.3	A	9	–	69	2.3	B	3	–	9	94.6		
49	39.8	A	10	–	66	9.8	B	6	–	21	76.2		
Experiment 17 – São José do Rio Pardo city													
0	35.8	A	21.7	–	69.0	35.4	A	22.3	–	68.0	–	Susceptible	28
3	46.1	A	17	–	89	34.6	A	18	–	77	24.2		
7	41.8	A	17	–	63	33.4	A	9	–	126	19.3		
14	35.0	A	21	–	65	1.0	B	0	–	5	97.1		
21	32.0	A	20	–	60	0.8	B	0	–	4	97.5		
28	34.2	A	20	–	59	0.7	B	0	–	5	97.9		
35	44.5	A	19	–	117	15.5	B	0	–	20	64.8		
42	61.0	A	20	–	139	37.0	B	0	–	128	38.7		
49	93.2	A	41	–	223	66.0	B	9	–	145	28.5		
Experiment 18 – Santo Antonio de Posse													
0	23.4	A	12.1	–	49.3	23.5	A	12.3	–	50.1	–	Susceptible	14
3	26.3	A	12	–	35	19.0	A	7	–	42	27.4		
7	19.9	A	14	–	46	15.4	B	5	–	34	22.3		
14	21.0	A	15	–	51	1.1	B	0	–	5	94.8		
21	19.0	A	13	–	39	13.8	B	3	–	65	27.8		
28	24.0	A	14	–	38	19.2	A	4	–	61	20.2		
35	26.0	A	15	–	39	23.5	A	9	–	78	10.2		
Experiment 19 – Vargem Grande do Sul													
0	40.8	A	20.7	–	67.3	40.6	A	20.3	–	68.1	–	Susceptible	42
3	42.9	A	23	–	65	21.5	B	12	–	45	49.6		
7	45.0	A	18	–	68	20.4	B	8	–	49	54.4		
14	46.9	A	21	–	71	1.2	B	0	–	3	97.4		
21	41.4	A	22	–	78	1.0	B	0	–	1	97.6		
28	38.2	A	21	–	56	0.0	B	0	–	0	100.0		
35	38.0	A	23	–	62	0.0	B	0	–	0	100.0		
42	44.0	A	23	–	65	3.1	B	0	–	18	92.9		
49	46.0	A	17	–	69	14.4	B	0	–	29	68.5		
56	52.0	A	18	–	57	32.0	B	3	–	78	38.1		
Experiment 20 – São Sebastião da Grama													
0	89.9	A	33.3	–	147.3	89.7	A	33.1	–	146.5	–	Susceptible	21
3	86.4	A	46	–	178	65.3	A	13	–	101	24.3		
7	101.5	A	41	–	198	71.3	B	17	–	112	29.7		
14	99.3	A	54	–	213	4.5	B	0	–	21	95.5		
21	98.7	A	55	–	206	3.2	B	0	–	14	96.8		
28	105.6	A	68	–	187	21.4	B	0	–	55	79.7		
35	110.4	A	72	–	198	43.1	B	1	–	85	60.9		

[1] Means values followed by the same letter on the same line do not differ significantly at a 95% reliability level.
[2] Commercial formulation purchased in the local market.[3] Rating susceptible performed based on the efficacy (>90%) using the arithmetic mean.
[3] Rating resistant performed based on the efficacy (≤90%) using the arithmetic mean.
[4] The last day of the study that the chemical compund show efficacy ≥90%.

first trial conducted with such molecule against *R. (B.) microplus* was performed by USDA researchers (Lohmeyer et al., 2012), which used different dosages (2.5 and 5.0 mg/kg) of an unregistered novaluron formulation. Results obtained by such authors reveal that both concentrations of pour on novaluron evaluated appear to have modest effects on southern cattle ticks. Therapeutically, both tested dosages caused a small reduction in numbers of recovered engorged female ticks, in comparison with amounts recovered from the untreated control group. In this case, Lohmeyer et al. (2012) described that some degree of residual efficacy was observed on the control of larvae that were applied to animals one and two weeks after their treatment. At the present study, results similar to those obtained by Lohmeyer et al. (2012) were observed for the novaluron (2.0 mg/kg) + eprinomectin (0.36 mg/kg) combination in all evaluated *R. (B.) microplus* strains. In none of these tick populations, the novel novaluron + eprinomectin association reached efficacy values superior to 50% against *R. (B.) microplus* females, measuring between 4.5 and 8.0 mm long, parasitizing bovines. Efficacy results obtained by this association on the present study are significantly inferior to indexes required by Brazilian legislation [Brazilian Ministry of Agriculture, Livestock and Food Supply (Ministério da Agricultura, Pecuária e Abastecimento – MAPA)] in order for a product to be licensed and commercialized as an acaricide in the country (Brasil, 1997). Such findings, allied to results obtained with this same molecule by Lohmeyer et al. (2012), reinforce the perception that maybe formulations of novaluron, in utilized dosages and administration routes, may not be an effective tool for controlling *R. (B.) microplus*. However, future studies must be performed in order to prove and reinforce such hypothesis.

Few studies were identified by present researchers, in consulted literature, which aimed to evaluate efficacy indexes of eprinomectin against *R. (B.) microplus* (Rangel, 2003; Aguirre et al., 2005; Lifschitz et al., 2016). Additionally, all studies report elevated efficacies (≥95%) of such active principle, administered topically (pour on), in different dosages, against the aforementioned ectoparasite. Results obtained by eprinomectin, administered via pour on

Table 4
Mean counts of female *R. (B.) microplus* (4.5–8.0 mm long) for control and treated cattle groups; results of variance analysis of tick counts and efficacy percentages in experiments performed in Midwest and South regions of Brazil (Mato Grosso do Sul, Goiás and Paraná States).

Day of study	T01: Control (Saline solution)					T02: Fluazuron (2.5 mg/kg)[2]					Efficacy (%)	Classification of *R. (B.) microplus* strain to fluazuron[3]	Residual efficacy of fluazrunon to *R. (B.) microplus*4
	Mean tick count[1]		Range			Mean tick count[1]		Range			Arithmetic mean		
Experiment 21 – Bandeirantes city (Mato Grosso do Sul State)													
0	59.3	A	18.0	–	127.33	59.5	A	17.3	–	129.0	–	Susceptible	35
3	46.8	A	13	–	72	56.8	A	43	–	101	0.0		
7	41.1	A	12	–	79	33.7	A	3	–	78	18.3		
14	30.9	A	14	–	63	6.9	B	1	–	35	77.8		
21	68.9	A	20	–	107	2.1	B	0	–	6	97.0		
28	19.3	A	17	–	22	0.8	B	0	–	3	95.9		
35	22.4	A	17	–	30	0.5	B	0	–	1	97.8		
42	39.1	A	14	–	66	26.8	A	7	–	162	31.7		
49	36.3	A	10	–	60	34.1	A	8	–	158	6.4		
Experiment 22 – Terenos city (Mato Grosso do Sul State)													
0	33.2	A	8.3	–	110.0	33.7	A	8.7	–	110.7	–	Resistant or with low susceptibility	Not applied
3	42.7	A	8	–	144	36.3	A	6	–	112	16.3		
7	64.1	A	9	–	214	34.1	B	7	–	90	47.6		
14	52.1	A	7	–	195	31.3	B	7	–	115	40.8		
21	22.4	A	8	–	80	13.7	B	0	–	45	39.7		
28	14.4	A	7	–	36	5.1	A	0	–	21	65.1		
Experiment 23 – Jaraguari city (Mato Grosso do Sul State)													
0	68.9	A	13.3	–	240.7	69.0	A	14.7	–	267.0	–	Resistant or with low susceptibility	Not applied
3	62.9	A	14	–	183	74.9	A	24	–	161	0.0		
7	68.4	A	9	–	145	74.2	A	12	–	234	0.0		
14	97.8	A	7	–	378	32.3	B	8	–	71	67.0		
21	105.7	A	16	–	274	19.0	B	2	–	71	82.1		
28	112.0	A	17	–	365	43.9	B	5	–	106	60.9		
35	101.7	A	10	–	317	115.2	A	30	–	342	0.0		
Experiment 24 – Caiapônia city (Goiás State)													
0	44.3	A	27.2	–	77.8	44.7	A	26.3	–	78.7	–	Susceptible	35
3	39.7	A	21	–	78	32.1	A	16	–	56	19.9		
7	44.5	A	24	–	119	29.8	B	15	–	43	33.6		
14	47.6	A	18	–	87	2.7	B	0	–	3	94.4		
21	51.3	A	21	–	91	1.1	B	0	–	5	97.9		
28	43.5	A	26	–	58	0.2	B	0	–	4	99.5		
35	44.3	A	23	–	67	0.3	B	0	–	3	99.3		
42	47.8	A	21	–	66	13.7	B	8	–	28	71.6		
49	49.7	A	20	–	69	17.6	B	8	–	32	64.9		
Experiment 25 – Jataí city (Goiás State)													
0	59.3	A	18.0	–	111.0	59.5	A	32.3	–	113.0	–	Susceptible	35
3	46.8	A	13	–	78	57.1	A	25	–	101	0.0		
7	41.1	A	12	–	78	33.7	A	3	–	74	18.3		
14	30.9	A	9	–	63	6.9	B	1	–	35	77.8		
21	68.9	A	20	–	105	0.9	B	0	–	6	98.7		
28	19.3	A	10	–	32	0.8	B	0	–	3	95.9		
35	22.4	A	15	–	30	0.7	B	0	–	2	96.9		
42	39.1	A	4	–	73	26.8	B	7	–	162	31.7		
Experiment 26 – Icaraíma city (Paraná State)													
0	93.9	A	35.3	–	145.7	92.4	A	35.7	–	148.7	–	Susceptible	28
3	159.1	A	32	–	156	110.5	A	21	–	101	29.4		
7	136.7	A	31	–	154	101.7	B	17	–	98	24.3		
14	133.2	A	30	–	143	0.0	B	0	–	5	99.8		
21	115.6	A	21	–	134	0.0	B	0	–	0	100.0		
28	109.5	A	23	–	121	0.0	B	0	–	0	100.0		
35	103.8	A	19	–	101	23.5	B	0	–	18	77.0		
42	93.7	A	19	–	98	40.9	B	4	–	78	55.6		
Experiment 27 – Umuarama city (Paraná State)													
0	21.0	A	13.3	–	44.2	21.7	A	11.3	–	46.7	–	Susceptible	42
3	24.8	A	15	–	50	20.6	A	14	–	32	19.6		
7	28.5	A	12	–	61	16.9	B	9	–	29	42.6		
14	27.3	A	12	–	66	2.2	B	0	–	7	92.2		
21	27.1	A	11	–	39	1.0	B	0	–	3	96.4		
28	26.1	A	14	–	43	1.5	B	0	–	6	94.4		
35	29.0	A	20	–	43	2.1	B	0	–	4	93.0		
42	31.5	A	18	–	54	2.3	B	0	–	8	92.9		
49	27.0	A	18	–	34	8.9	B	5	–	14	68.1		
56	25.1	A	15	–	43	16.7	B	12	–	23	35.6		

[1] Means values followed by the same letter on the same line do not differ significantly at a 95% reliability level.
[2] Commercial formulation purchased in the local market.
[3] Rating susceptible performed based on the efficacy (>90%) using the arithmetic mean.
[3] Rating resistant performed based on the efficacy (≤90%) using the arithmetic mean.
[4] The last day of the study that the chemical compund show efficacy ≥90%.

Table 5
Mean counts of female *R. (B.) microplus* (4.5–8.0 mm long) for control and treated cattle groups; results of variance analysis of tick counts and efficacy percentages in experiments performed in Midwest and Southeast regions of Brazil (São Paulo and Goiás State).

Day of study	T01: Control (Saline solution)					T03: Novaluron (2.0 mg/kg) + Epinomectin (0.36 mg/kg)[2]					T02: Fluazuron (2.5 mg/kg)[2]					T02: Eprinomectin (0.5 mg/kg)[2]					Efficacy (%)			Classification of *R. (B.) microplus* strain			Residual efficacy of fluazrunon to *R. (B.) microplus*4
	Mean tick count[1]		Range			Mean tick count[1]		Range			Mean tick count[1]		Range			Mean tick count[1]		Range			Novaluron + Eprinomectin	Fluazuron	Eprinomectin	Novaluron + Eprinomectin3	Fluazuron[3]	Eprinomectin[3]	
Experiment 28 – Espírito Santo do Pinhal city (São Paulo State)																										
0	33.8	A	20.7	–	67.3	33.7	A	20.1	–	66.3	33.6	A	20.3	–	66.3	33.5	A	20.0	–	66.8	–	–	–	Resistant or with low susceptibility	Susceptible	Resistant or with low susceptibility	42
3	35.9	A	18	–	68	33.9	A	17	–	63	29.5	A	14	–	56	34.5	A	21	–	54	5.3	17.3	3.0				
7	38.0	A	21	–	61	30.7	A	9	–	32	27.7	A	11	–	45	26.5	A	9	–	21	19.0	26.6	29.6				
14	39.9	A	22	–	78	24.0	B	5	–	33	0.7	C	0	–	5	16.7	B	3	–	29	39.7	98.2	57.8				
21	34.4	A	21	–	56	20.2	B	2	–	33	0.0	C	0	–	0	21.5	B	4	–	36	41.1	100.0	36.9				
28	31.2	A	24	–	62	19.3	B	4	–	32	0.1	C	0	–	1	23.4	B	7	–	43	38.0	99.7	24.3				
35	31.0	A	21	–	65	20.3	B	9	–	32	1.3	C	0	–	5	25.6	B	12	–	41	34.3	95.8	16.7				
42	37.0	A	21	–	69	20.6	B	7	–	34	3.2	C	0	–	8	41.5	A	23	–	61	44.2	91.3	0.0				
49	39.0	A	18	–	57	24.7	B	9	–	37	8.5	C	0	–	13	39.8	A	18	–	65	36.5	78.1	0.0				
56	45.0	A	28	–	61	27.2	B	4	–	48	26.7	B	1	–	56	45.7	A	24	–	68	39.4	40.3	0.0				
Experiment 29 – São João da Boa Vista city (São Paulo State)																										
0	62.5	A	20.3	–	203.0	62.9	A	21.7	–	204.3	62.4	A	20.1	–	205.6	62.3	A	21.0	–	201.7	–	–	–	Resistant or with low susceptibility	Susceptible	Resistant or with low susceptibility	21
3	73.1	A	39	–	215	63.0	A	18	–	201	61.2	A	18	–	187	64.5	A	21	–	197	14.3	16.2	11.5				
7	81.3	A	31	–	209	54.7	B	9	–	139	58.7	B	15	–	132	45.7	B	9	–	123	33.1	27.7	43.6				
14	94.7	A	45	–	198	55.2	B	15	–	117	2.3	C	0	–	7	38.7	B	14	–	98	42.1	97.6	59.0				
21	95.8	A	43	–	201	58.5	B	12	–	132	1.3	C	0	–	5	49.9	B	16	–	110	39.3	98.6	47.8				
28	102.0	A	25	–	204	64.5	B	9	–	138	20.1	C	0	–	45	61.4	B	11	–	169	37.1	80.3	36.9				
35	104.3	A	32	–	201	71.6	B	16	–	136	45.6	C	4	–	67	83.8	B	19	–	187	31.8	56.2	19.4				
Experiment 30 – Tambaú city (São Paulo State)																										
0	79.2	A	23.7	–	213.3	79.6	A	21.7	–	212.7	79.1	A	22.3	–	211.7	78.8	A	22.1	–	212.3	–	–	–	Resistant or with low susceptibility	Susceptible	Resistant or with low susceptibility	35
3	87.5	A	23	–	256	65.7	B	17	–	202	81.5	A	24	–	244	60.1	B	21	–	212	25.3	6.8	31.0				
7	84.5	A	28	–	244	59.7	B	15	–	198	78.9	A	21	–	232	72.3	B	19	–	231	29.7	6.5	14.0				
14	93.2	A	32	–	267	61.4	B	13	–	141	3.4	C	3	–	7	67.8	B	15	–	167	34.4	96.3	26.9				
21	101.7	A	31	–	256	65.3	B	14	–	132	5.4	C	1	–	8	98.9	A	23	–	239	36.1	94.7	2.3				
28	112.2	A	33	–	298	71.3	B	11	–	145	4.3	C	0	–	12	105.4	A	32	–	278	36.7	96.2	5.6				
35	108.3	A	37	–	302	78.7	B	10	–	149	7.6	C	0	–	16	112.3	A	31	–	278	27.7	93.0	0.0				
42	110.5	A	35	–	298	81.2	B	14	–	184	12.6	C	1	–	25	123.7	A	30	–	298	26.9	88.6	0.0				
49	115.2	A	37	–	313	84.6	B	16	–	189	23.5	C	3	–	35	119.8	A	32	–	309	26.9	79.6	0.0				
Experiment 31 – Jataí city (Goiás State)																										
0	49.5	A	25.3	–	123.7	49.9	A	25.7	–	122.3	49.4	A	25.7	–	121.6	49.3	A	25.3	–	122.7	–	–	–	Resistant or with low susceptibility	Susceptible	Resistant or with low susceptibility	35
3	46.7	A	23	–	145	43.5	A	20	–	101	41.6	A	21	–	118	40.2	A	21	–	101	7.5	10.8	13.5				
7	44.3	A	22	–	149	29.4	B	15	–	78	32.7	A	18	–	101	38.7	A	18	–	113	34.1	26.1	12.3				
14	41.4	A	21	–	141	28.6	B	13	–	67	0.3	C	0	–	4	27.6	B	8	–	72	31.4	99.3	33.1				
21	48.9	A	18	–	156	28.7	B	12	–	64	0.0	C	0	–	0	31.7	B	9	–	78	41.7	100.0	34.9				
28	49.3	A	16	–	165	29.1	C	11	–	56	3.5	D	0	–	7	39.8	B	8	–	76	41.4	92.9	18.9				
35	50.2	A	18	–	178	28.7	B	9	–	49	4.7	C	0	–	16	45.7	A	12	–	143	43.2	90.6	8.6				
42	52.1	A	17	–	179	32.5	B	9	–	58	24.5	C	1	–	34	46.9	A	11	–	155	38.1	52.9	9.6				
49	47.8	A	21	–	145	31.4	B	8	–	73	34.6	B	9	–	101	44.5	A	18	–	123	34.8	27.5	6.5				
Experiment 32 – Acreúna city (Goiás State)																										
0	25.6	A	14.3	–	44.7	25.3	A	14.7	–	44.1	25.6	A	14.4	–	44.5	25.3	A	14.8	–	44.1	–	–	–	Resistant or with low susceptibility	Susceptible	Resistant or with low susceptibility	21
3	26.7	A	15	–	43	19.3	A	14	–	32	18,4	A	13	–	36	21.4	A	13	–	37	26.9	31.1	18.9				
7	32.1	A	16	–	47	16.7	B	8	–	30	16.5	C	9	–	31	15.4	B	7	–	28	47.7	48.6	51.5				
14	28.7	A	13	–	39	16.9	B	6	–	29	1.4	C	0	–	7	15.9	B	1	–	12	40.4	95.1	43.9				
21	30.2	A	16	–	41	16.9	B	7	–	31	1.6	C	0	–	12	20.5	B	9	–	34	43.4	94.7	31.3				
28	30.7	A	15	–	46	19.3	B	6	–	27	11.3	C	0	–	31	23.5	B	12	–	43	36.4	63.2	22.5				
35	34.5	A	15		49	21.4	B	7	–	32	17.8	C	9		45	26.7	B	11		59	37.2	48.4	21.7				

[1] Means values followed by the same letter on the same line do not differ significantly at a 95% reliability level.
[2] Commercial formulation purchased in the local market.
[3] Rating susceptible performed based on the efficacy (>90%) using the arithmetic mean.
[3] Rating resistant performed based on the efficacy (≤90%) using the arithmetic mean.
[4] The last day of the study that the chemical compund show efficacy ≥90%.

the dosage of 0.5 mg/kg, described here, make it clear that all populations of *R. (B.) microplus* tested on the present study are resistant, or present low susceptibility, to such active component.

Based on all results obtained using 32 field efficacy trials with fluazuron (2.5 mg/kg), as well as five experiments with novaluron (2.0 mg/kg) + eprinomectin (0.36 mg/kg) and eprinomectin (0.5 mg/kg), it is possible to conclude that four southern cattle tick populations were diagnosed as resistant, or at least less susceptible, to fluazuron (2.5 mg/kg). Such fact was detected in farms where owners administered this compound in their herds for a minimum period of five years, with treatment intervals of 30 to 55 days during the rainy season. However, *in vitro* studies should be performed in order to reinforce the results obtained in the present study. Regarding efficacies obtained by the novel molecule novaluron, allied to eprinomectin, against such ixodidae, this combination showed efficacy values inferior to 48.0% in all trials. These findings, allied to results obtained with the same molecule by Lohmeyer et al. (2012), reinforce the perception that perhaps novaluron formulations, in tested dosages and administration routes, may not be effective tools for controlling *R. (B.) microplus*. Besides, all five *R. (B.) microplus* strains tested were diagnosed as resistant, or with low susceptibility, to eprinomectin (0.5 mg/kg). Even though fluazuron, administered via pour on, is still an excellent principle used for controlling *Rhipicephalus (Boophilus) microplus,* resistance management strategies should be quickly implemented in order to keep the selection pressure for this compound at a minimum level in Brazil.

Conflicts of interest

All authors declare no conflicts of interest.

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