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Toxicity interaction of fipronil and imidacloprid against *Coptotermes formasanus*

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**TOXICITY INTERACTION OF FIPRONIL AND IMIDACLOPRID AGAINST
*COPTOTERMES FORMOSANUS***

A Thesis
Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Entomology

By
Pan Luo
B.S., China Agricultural University, 2008
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ABSTRACT

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Isoptera: Rhinotermitidae), is considered one of the most destructive structural pests in the world, especially in warm and humid areas. Insecticide application is an effective strategy in termite control. In recent years, non-repellent insecticides have become popular for their high efficacy due to delayed toxicity and horizontal transfer. Fipronil (registered name Termidor®) and imidacloprid (registered name Premise®) have been applied to the perimeter of millions of houses in the United States. Fipronil and imidacloprid have different modes of action which may produce a synergistic effect when combined. There have been no studies on the toxicity interaction of fipronil and imidacloprid against termites including the Formosan subterranean termite.

The original objective of the research was to determine whether combinations of the termiticides lead to enhanced toxicity against Formosan subterranean termites. Combinations of the non-repellent insecticides were treated on filter paper and sand for evaluation. After timed exposures, any living termites were transferred to untreated Petri dishes. Mortality of termites was recorded before and after the transfer. Lower mortality was observed when imidacloprid was mixed with fipronil compared to fipronil alone. Mortality was increased by the mixture over imidacloprid alone. To validate these results, more combinations were introduced in the second

and third set of experiments. Besides the recording of mortality, the number of excavation holes made by termites in sand was also counted to determine whether excavation activity was related to mortality effects. A second objective was to seek a threshold level whereby the efficacy of fipronil becomes negatively impacted by imidacloprid presence. A threshold of between 15 and 25 ppm imidacloprid added to 100 ppm fipronil reduced the efficacy of fipronil. An increase in the number of excavation holes was significantly associated with a rising mortality, indicating imidacloprid affected the uptake of fipronil by reducing termite excavation behavior of treated soil. In practical terms and of potential concern for homeowners, the studies suggest that the efficacy of Termidor® applied around the perimeters of houses may be negatively affected by the presence of Premise®.

CHAPTER 1

INTRODUCTION

Termites belong to the order Isoptera and are some of the most important structural pests. According to the classification proposed by Snyder (1949) and Emerson (1955), there are six families of Isoptera in the world: Mastotermitidae, Kalotermitidae, Hodotermitidae, Rhinotermitidae, Serritermitidae and Termitidae. Four families have been described in the United States: Kalotermitidae, Hodotermitidae, Rhinotermitidae and Termitidae (Snyder 1949, Emerson 1955). There are forty to fifty species of termites distributed in the United States; however, only two drywood termites, *Cryptotermes brevis* (Walker) and *Incisitermes minor* (Hagen), one “tree” termite, *Nasutitermes costalis* (Holmgren) and five subterranean termites, *Coptotermes formosanus* Shiraki, *C. gestroi* (Wasmann), *Reticulitermes hesperus* Banks, *R. flavipes* (Kollar) and *R. virginicus* (Banks), are generally considered important economic pests (Su and Scheffrahn 1990, Culliney and Grace 2000, Scheffrahn et al. 2002, Cabrera et al. 2005). The economic loss due to termites has been estimated at 11 billion dollars per year and has increased as the standard of living has improved (Su 2002, Oi et al. 2003). Among these termite species, *C. formosanus* is known as the most destructive species in the United States because it is the most aggressive and has larger colony populations; however, due to its wide distribution in the U.S., *R. flavipes* is regarded the single most economically important species (Su and Scheffrahn 1990, Culliney and Grace 2000).

Coptotermes formosanus is sometimes called the Oriental subterranean termites or Asian subterranean termites indicating its origin (Su and Tamashiro 1987). The first record of *C. formosanus* in the continental United States was from a shipyard in Houston, Texas in 1965 (Beal 1967). It was reported that Formosan subterranean termites caused billions of dollars in damage and control costs annually nationwide, with 300 million dollars in property damage, preventive measures and structural repair in New Orleans alone (USDA-ARS 2007).

Insecticide application is an effective strategy for termite control (Gold et al. 1996, Henderson 2003). Fipronil was first introduced to the United States in 1996 by Rhone Poulenc Ag. Company (U.S. EPA Office of Prevention, Pesticide and Toxic Substances 1996a). It is used as a termite control product, for fire ants, cockroaches, turf insects and fleas (U.S. EPA Office of Prevention, Pesticide and Toxic Substances 1996b, Cox 2005). In addition, it is applied to crops such as corn and cotton for plant protection (Overmyer et al. 2005). The first fipronil product in termite control in the United States was Termidor® which was approved by the EPA in 1999 (PANNA 2009). Compared to fipronil, imidacloprid has a longer history- its first synthesis and mode of action were reported in 1984 (Schroder and Flattum 1984). Premise® is the first trademark of the imidacloprid product for termite control in the United States marketed by Bayer Corporation in the mid-1990s. Besides its uses in termite, ant and flea control, it is also applied

to agricultural products to control sucking and chewing insect species. Its application formulations include foliar sprays, soil treatments and seed dressings (Elbert et al. 1998).

Fipronil belongs to the phenylpyrazole class of insecticide (IRAC 2008). It is known to inhibit the neurotransmitter γ -aminobutyric acid (GABA) in both insects and vertebrates (Hosie et al. 1995, Tingle et al. 2003). The exact binding sites are still unknown (Le Corrionc et al. 2002). Recently glutamate-gated chloride channels (GluCl), chloride channels unique to insects, were also found to be inhibited by fipronil (Ikeda et al. 2003). The mode of action may play a critical role in the high selective toxicity of fipronil in insects but not mammals (Zhao et al. 2004).

Imidacloprid directly binds to postsynaptic nicotinic acetylcholine receptors (nAChR) which causes its toxic effects in insects as well as vertebrates (Elbert et al. 1998). It is the first insecticide found to block nAChR completely and irreversibly in insects; the binding is 1,000 times stronger in insects than in vertebrates which endows its high insect selectivity (Methfessel 1992).

Fipronil and imidacloprid, as non-repellent insecticides, have attracted more interest than traditional repellent insecticides (Kard 2001, Henderson 2003, Ibrahim et al. 2003, Shelton and Grace 2003). Non-repellent insecticides often maintain the property of non-repellency even at high concentrations (up to 500 part per million in *Reticulitermes hesperus*) (Saran and Rust 2007). The transfer of fipronil and imidacloprid among termite workers as well as between

workers and soldiers has been studied (Thorne and Breisch 2001, Ibrahim et al. 2003, Saran and Rust 2007). There is a linear relationship between dose uptake and insecticide contact time in subterranean termites (Saran and Rust 2007). Body contact (including grooming) plays the main role in lethal dose horizontal transfer compared to the transmission by trophallaxis (Saran and Rust 2007). The transfer from soldiers to workers is significantly higher than workers to soldiers (Ibrahim et al. 2003). A study on distance of horizontal transfer in the field showed that the lethal effects in Formosan subterranean termites may be limited however (Su 2005).

Since Termidor® and Premise® represent two of the most popular termiticides in the termite control market it is common for pest control companies treating the perimeter of houses to use either of them. If the owners of a house changes hands, or if pest control contracts are shifted from one company to another, a house might be multi-treated over time with Termidor® and Premise®. This would suggest that a mixture of Premise® and Termidor® would likely occur in the field. However, there have been no studies on the toxicity interaction of fipronil and imidacloprid against Formosan subterranean termites.

I hypothesized that there was toxicity interaction effects between fipronil and imidacloprid and that termite behavior may play an important role in their combined toxicity effects. In this thesis studies on termite survivorship to different ratios of combinations of fipronil and imidacloprid were conducted. In addition, termite behavioral response to the

toxicants was observed and possible explanations of changes in survivorship due to different combinations are discussed.

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CHAPTER 2

CHANGES IN SURVIVORSHIP OF *COPTOTERMES FORMOSANUS* DUE TO DIFFERENT COMBINATIONS OF FIPRONIL AND IMIDACLOPRID

Introduction

Both fipronil and imidacloprid have become popular for their non-repellent and efficacious nature (Su 2005, Rust and Saran 2008). The features of non-repellency, delayed toxicity and horizontal transfer, lead to higher control efficacy for termite populations than do traditional repellent pyrethroids or acutely toxic organophosphorous termiticides (Kard 2001, Wagner 2003, Hu 2005, Tsunoda 2006). Fipronil inhibits the neurotransmitter γ -aminobutyric acid (GABA) and glutamate-gated chloride channels (GluCl) while imidacloprid binds to the postsynaptic nicotinic acetylcholine receptors (nAChR) (Abbink 1991, Hosie et al. 1995, Ikeda et al. 2003, Tingle et al. 2003). Different modes of action indicate synergistic effects may exist if both compounds are added together.

As these compounds represent two of most common termiticides in the termite control market today, numerous studies have been conducted on the toxicity of both fipronil and imidacloprid in the laboratory and field (Ibrahim et al. 2003, Osbrink et al. 2005, Su 2005). However, it is surprising that no research has been conducted on their toxicity interaction. Laboratory experiments were conducted to test mortality in the Formosan subterranean termite *Coptotermes formosanus* Shiraki to fipronil, imidacloprid and their combinations. Mortality was also evaluated when termites were transferred to untreated Petri dishes following their survival in termiticide treatments. The objective of the experiment was to determine whether there was

synergism or not between fipronil and imidacloprid against field-collected Formosan subterranean termites in laboratory arenas.

Materials and Methods

Termites. Formosan subterranean termites, *C. formosanus* were collected using a crate-trapping technique (Smith et al. 2004, Gautam and Henderson 2010) from Brechtel Park in New Orleans, Louisiana in October, 2008. The crate trap was kept in a 140-L trash can with a lid and was stored in the urban entomology laboratory under room conditions (26 - 28°C, 70-80% RH). Prior to the start of the trials, healthy and active workers and soldiers were transferred from a crate trap (one colony) to a plastic container with moist brown paper towels (Tork Universal hand towel, SCA Tissue North America, Neenah, WI). One colony was used in the bioassay.

Termiticides. The two termiticides tested in the trials were fipronil (Fipronil Tech., BASF 350I, BASF Corporation, Research Triangle Park, NC) and imidacloprid (Premise® 75, Bayer Corporation, Kansas City, MO).

Bioassays. Fifty grams of autoclaved dry sand (construction sand, Louisiana Cement Products, LLC., Baton Rouge, LA) were loaded in each Petri dish (100 mm × 15 mm, Medegan Medical Products, Gallaway, TN) and a filter paper (55mm in diameter, Grade 1 and Grade 2, Whatman) was placed on top of the sand. Termiticide solutions were prepared by dissolving pre-weighed termiticides with water in 100 ml volumetric flasks. In order to ensure fipronil was

mixed in water completely (no visible particles), the volumetric flasks were placed in an ultrasonic bath (Bransonic 1510R-MT ultrasonic cleaner, Danbury, CT) working at 70 W and 42 kHz for 20 - 30 minutes. The four termiticide solutions prepared for the bioassay and the concentrations of each termiticide in the substrate are shown in Table 2.1. A 10 ml solution was added to the filter paper in the Petri dishes and 10 ml water only was added as the control.

Table 2.1. List of treatments and their concentrations in solutions and substrates.

Treatments	Concentrations in solutions (ppm)	Concentrations in substrates (ppm)
100 ppm F*	100 F	16.67 F
100 ppm F + 100 ppm I*	100 F + 100 I	16.67 F + 16.67 I
50 ppm F + 50 ppm I	50 F + 50 I	8.33 F + 8.33 I
100 ppm I	100 I	16.67 I
Control	0	0

*F: fipronil; I: imidacloprid.

Fifty termites, (45 workers and 5 soldiers) were introduced from the plastic container aforementioned to each treated Petri dish by a soft fine brush. Then every Petri dish was sealed with Parafilm® (Parafilm M, Pechiney Plastic Packaging, Chicago, IL) and stored in an isolated chamber at room temperature (21 – 23°C) (Fig. 2.1).

Each treatment was tested at four exposure times, 24, 41, 51 and 65 hours and two replicates were performed. Two Petri dishes of each treatment were randomly taken out of the chamber at each time period. The number of surviving termites, defined as any part of the termite moving with or without the stimulus from a brush was recorded.

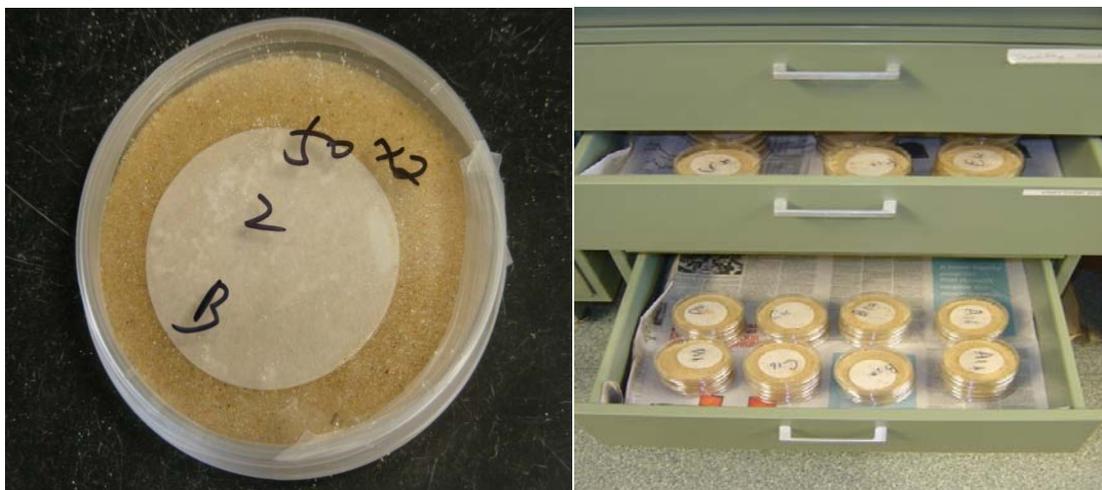


Fig. 2.1. A sealed Petri dish (left) and the storage drawer (right).

After counting surviving termites, they were transferred using clean forceps to untreated Petri dishes (100 mm × 15 mm, Medegan Medical Products, Gallaway, TN) which were pre-loaded with 50 g autoclaved dry sand, a filter paper (55mm in diameter, Grade 1 and Grade 2, Whatman) and 10 ml water. All toxicant-free Petri dishes were labeled with the termiticide termites were previously exposed to and the exposure time (24, 41, 51 or 65 hours). They were then stored back in the dark drawer at room temperature (21 – 23°C). Surviving termites in Petri dishes were examined again for mortality between 24 hours and 52 hours after placement into untreated dishes.

Data analysis. A two-way ANOVA was used to analyze the variance of survival numbers among different concentration combinations and different times by using a generalized linear model (PROC GLM) in SAS software (SAS 9.1, SAS Institute, NC). The means of survival numbers were separated using a Least Significant Difference test (LSD) ($\alpha=0.05$).

In the arenas where termites were transferred to untreated Petri dishes, a two-way ANOVA was used to analyze the variance of mortality percentages (= Dead termite numbers / Total termite numbers transferred) in different treatments for each time. The means of mortality percentages were separated by LSD ($\alpha=0.05$).

Results

There was a significant interaction between fipronil and imidacloprid. In addition, a significant interaction between treatment and time was found ($F = 9.83$; $df = 12, 39$; $P < 0.0001$).

Treatment effect. There was a significant difference in survival numbers among the treatments ($F = 214.04$; $df = 4, 39$; $P < 0.0001$) (Figure 2.2). Among the four different termiticides treatments, fipronil alone caused the lowest survival while imidacloprid alone resulted in the highest survival numbers at 24, 41, 51 and 65 hours. The survival numbers in the treatment of 100 ppm fipronil plus 100 ppm imidacloprid were significantly lower than in the treatment of 100 ppm imidacloprid but significantly higher than in the treatment of 100 ppm fipronil at 41, 51 and 65 hours. There was no significant difference in survival numbers between treatments of 100 ppm imidacloprid and control at 24, 41 and 51 hours (Figure 2.2).

Time effect. There was a significant difference in survival numbers was found among observation times ($F = 54.63$; $df = 3, 39$; $P < 0.0001$) (Fig. 2.3). The survival numbers were significantly reduced in the treatment of 100 ppm fipronil as early as 24 hours, while there was

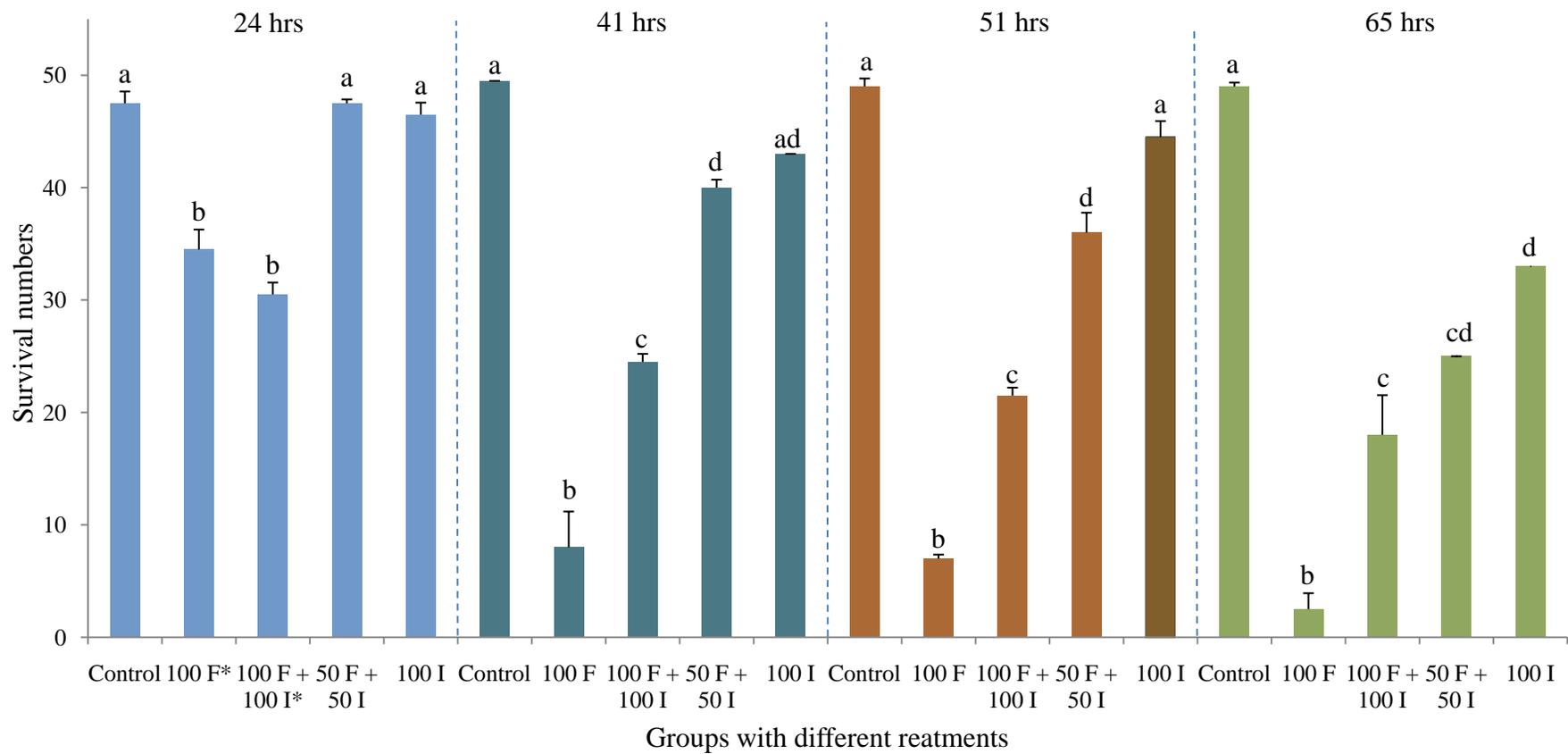


Fig. 2.2. Survival numbers in different treatments at different times. Means with the same letter are not significantly different.

* F: fipronil; I: imidacloprid.

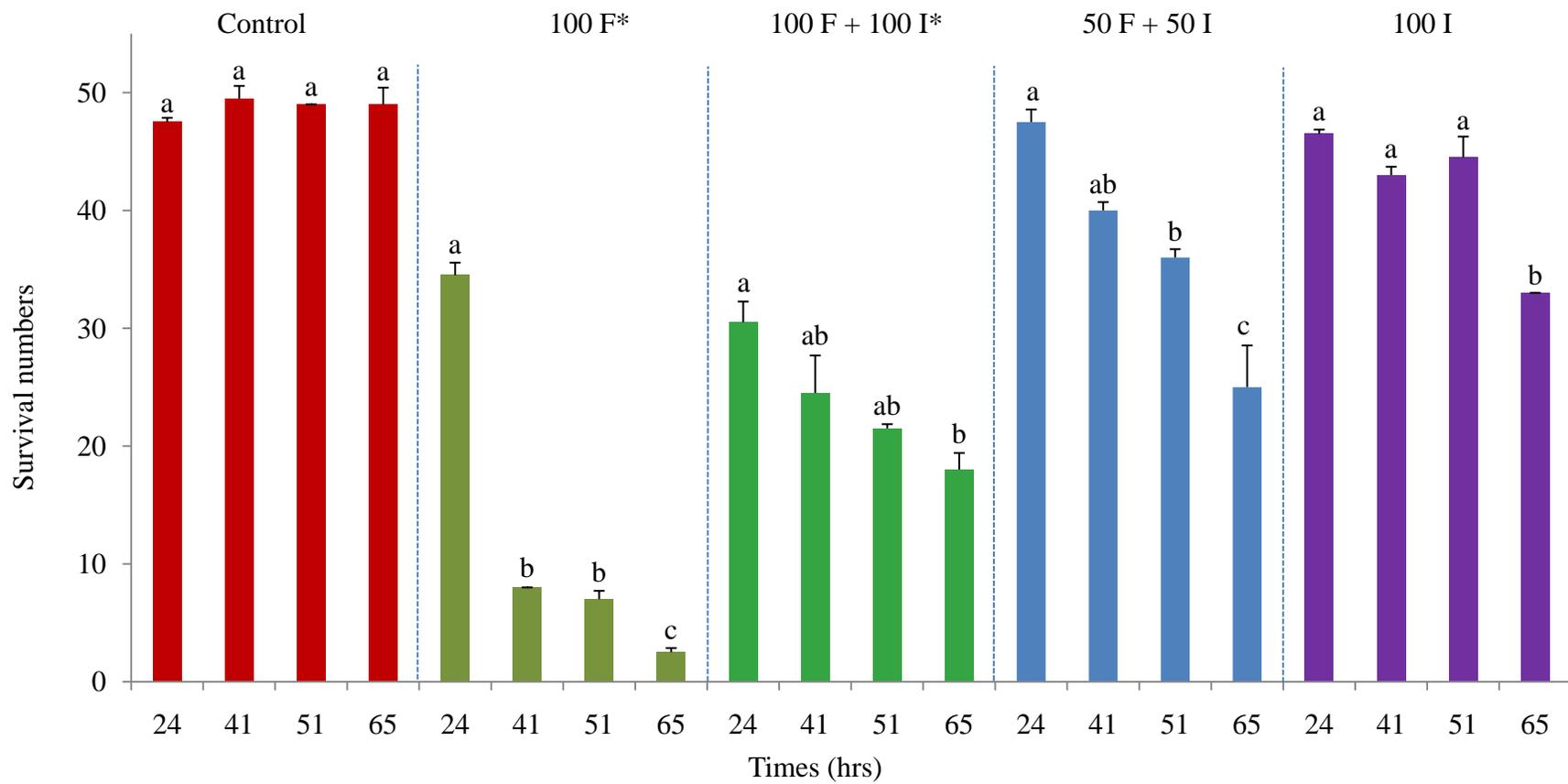


Fig. 2.3. Survival numbers in each treatment at different times. Means with the same letter are not significantly different.

* F: fipronil; I: imidacloprid.

no significant difference in the treatments of 100 ppm imidacloprid up to 51 hours (Fig. 2.3). The changes in survival numbers in the treatment of 100 ppm fipronil plus 100 ppm imidacloprid among different times were not significantly different ($F = 3.65$; $df = 3, 7$; $P = 0.1216$). There was no significant difference in survival numbers in the control ($F = 0.46$; $df = 3, 7$; $P = 0.7243$) (Fig. 2.3). In addition, some termites were heavily infected with fungi, especially when they were exposed to termiticides for a long time (i.e. 65 hours).

In the transfer study, termites previously treated with combinations of 100 ppm fipronil plus 100 ppm imidacloprid and 50 ppm fipronil plus 50 ppm imidacloprid for 24, 41 and 51 hours showed higher percentage mortality than those in the treatment of 100 ppm imidacloprid alone (Table 2.2). The mortality in treatments of 100 ppm fipronil plus 100 ppm imidacloprid with 41 hours or 51 hours exposure time and after around 50 hours in untreated Petri dishes was much higher than in treatments with 24 hours exposure time. A similar trend was also found in the treatment of 100 ppm imidacloprid alone (Table 2.2).

Discussion

Compared to the high mortality led by fipronil, the toxicity of imidacloprid was both reduced and delayed. One of the most interesting results found in the study was that mortalities in the combinations of fipronil and imidacloprid were lower than in fipronil alone but higher than in imidacloprid alone. These results caused me to reject the hypothesis that synergistic effects

Table 2.2. Mean percentage of mortality (\pm SEM) of termites in untreated arenas.

Exposure time in the previous treatment (hrs)	Time in the non-toxic Petri dishes	Previous treatments	Percentage of mortality (mean \pm SEM) (%)
24	30	Control	7.184 \pm 3.0165 a*
		100 ppm F	89.775 \pm 1.895 b
		100 ppm F+ 100 ppm I	14.447 \pm 3.733 a
		50 ppm F+ 50 ppm I	60.914 \pm 4.392 c
		100 ppm I	11.818 \pm 9.480 a
$F = 143.89$; $df = 4,9$; $P < 0.0001$			
41	52	Control	6.066 \pm 0.184 a
		100 ppm F	81.250 \pm 6.250 b
		100 ppm F+ 100 ppm I	95.000 \pm 5.000 b
		50 ppm F+ 50 ppm I	77.737 \pm 9.444 b
		100 ppm I	71.485 \pm 23.755 b
$F = 8.42$; $df = 4,9$; $P = 0.0191$			
51	52	Control	5.100 \pm 5.102 a
		100 ppm F	91.665 \pm 8.335 b
		100 ppm F+ 100 ppm I	86.150 \pm 4.330 bc
		50 ppm F+ 50 ppm I	93.165 \pm 3.975 b
		100 ppm I	56.054 \pm 17.760 c
$F = 15.74$; $df = 4,9$; $P = 0.0049$			

* Within each time period, means in the same column followed by different letters indicated significant differences between treatments. Means were separated by Least Significant Difference test (LSD) ($\alpha=0.05$)

existed between fipronil and imidacloprid. The result that imidacloprid alone led to the lowest mortalities among the four termiticide treatments and that no significant differences between treatments of imidacloprid and controls were observed indicated a low toxicity of imidacloprid in general (Fig. 2.1). This pharmacodynamic pattern of imidacloprid on termites was also noted in

other studies. For example, Thorne and Breisch (2001) observed that the effect of imidacloprid was so negligible that the symptoms would disappear if termites were not exposed to imidacloprid for a sufficient amount of time. Due to the relatively low toxicity of imidacloprid it was not surprising that the mortality in the combinations would be increased when fipronil was added. Thorne and Breisch (2001) also noticed delayed toxicity of imidacloprid and reported that death caused by imidacloprid took several days. In addition, Haagsma (2003) reported that complete mortality of *Reticulitermes hesperus* Banks required 14 days when they were exposed to 500 ppm imidacloprid for 2 hours. Therefore in the present study, it was not surprising that both the increase of time exposure to the termiticides and the time in untreated Petri dishes caused mortalities in chemical combinations and imidacloprid alone to increase (Table 2.2).

Although imidacloprid showed reduced and delayed toxicity, behavioral changes caused by imidacloprid were apparent and much faster than in other treatments. Immobility was noticed in termites either treated with imidacloprid alone or combinations of fipronil and imidacloprid as early as two hours posttreatment (unreported results from a preliminary experiment). Henderson (2003) reported that significantly decreased digging and walking behaviors were found when Formosan subterranean termites were exposed to imidacloprid at 9 hours, while the effect of fipronil on digging and walking behaviors was not significantly different from control groups.

Experiments on *Reticulitermes virginicus* (Banks) also showed immobility symptoms caused by imidacloprid as early as 4 hours posttreatment (Thorne and Breisch 2001).

It was reported that both fipronil and imidacloprid were mainly taken up and transferred by body contact (Haagsma 2003, Saran and Rust 2007). Since imidacloprid greatly reduces termite walking and digging behavior, the uptake and transfer of the toxicant may be inhibited (Henderson 2003). I propose that the presence of imidacloprid reduces the chance of termites coming in contact with and taking up the more toxic fipronil, leading to lower mortalities when imidacloprid and fipronil are combined.

More fungus was found in Petri dishes treated with imidacloprid and high mortalities were accompanied with this fungal association. It was reported that termites reduced removal of fungal spores when in the presence of imidacloprid, resulting in susceptibility to entomopathogenic fungi (Ramakrishnan et al. 1999). This feature of imidacloprid could be a complimentary character for the low toxicity.

In both directly treated and untreated arenas after exposure, the treatment with fipronil alone yielded a higher mortality than the other treatments (Fig. 2.2, Table 2.2). The treated arenas tested the direct effects of fipronil on termites, while untreated arenas were used to check the effects of fipronil-residue on termites. Since fipronil is a non-repellent termiticide, termites may enter treated areas inadvertently. After exposure to termiticides having delayed toxicity they

could continue to forage for an extended period of time. The results from untreated arenas indicated that termites that initially escaped death in treated arenas eventually died.

When the toxicity of fipronil in this study was compared with other publications, a lower level of mortality was noted. For example, Bagnères et al. (2009) reported that 77% and 90% mortality in American and French *Reticulitermes flavipes* (Kollar) was obtained when they were exposed to 1 ppm fipronil in sand after 24 hours, while this study showed 16 ppm (the concentration in the sand and filter paper when 100 ppm fipronil solution was added) yielded only 30% mortality of *C. formosanus* at 24 hours (Fig. 2.2). The difference in the mortality of *R. flavipes* and *C. formosanus* could be linked to the variation in susceptibility in the two species – *R. flavipes* is considered more susceptible than *C. formosanus* to fipronil (Remmen and Su 2005).

Although the results showed imidacloprid reduced the efficacy of fipronil and fipronil enhanced the toxicity of imidacloprid, the experimental design could be challenged by the limited concentrations in the bioassay - only two concentrations (100 ppm and 50 ppm) of fipronil and imidacloprid were tested. More combinations of fipronil and imidacloprid at different concentrations are needed to validate the results. Also in the transfer study I varied the time in my evaluation of toxicity between treatments. Observation time in the untreated Petri dishes should have used a constant time.

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CHAPTER 3

CHANGES IN SURVIVORSHIP OF *COPTOTERMES FORMOSANUS* DUE TO GRADIENT COMBINATIONS AND LOW-CONCENTRATION COMBINATIONS OF FIPRONIL AND IMIDACLOPRID

Introduction

Coptotermes formosanus Shiraki is one of the most destructive structural pests in the United States (Su and Scheffrahn 1990). Louisiana is a heavily infested state. For example, the economic loss caused by Formosan subterranean termites is estimated at 300 million dollars in New Orleans alone (USDA-ARS 2007). Two of most popular insecticides in the termite control market, fipronil and imidacloprid have been exhaustively studied for uptake by individuals as well as horizontal transfer within a colony (Baskaran et al. 1999, Ramakrishnan et al. 2000, Ibrahim et al. 2003, Osbrink et al. 2005, Su 2005, Tsunoda 2006, Tomalski et al. 2010). Studies using ^{14}C revealed that contact was the main pathway for the uptake of fipronil and imidacloprid and cuticular transport was necessary for toxicant transfer in a colony (Haagsma 2003, Saran and Rust 2007, Bagnères et al. 2009).

In laboratory bioassays, fipronil and imidacloprid showed non-repellency and delayed toxicity (Haagsma 2003, Ibrahim et al. 2003, Remmen and Su 2005, Saran and Rust 2007). Studies on behavior changes to termiticides showed that imidacloprid significantly inhibited termite digging, tunneling and walking behaviors (Thorne and Breisch 2001, Haagsma 2003, Henderson 2003). A soil penetration study showed concentration and thickness of treated substrates affected termite mortality (Hu 2005). Efficacy and degradation of fipronil and imidacloprid in the field were also reported (Baskaran et al. 1999, Osbrink et al. 2005, Su 2005).

Though fipronil and imidacloprid share delayed toxicity and non-repellency which make them successful in today's termite control market, they have different modes of action. Fipronil binds to GABA receptors and glutamate-gated chloride channels while imidacloprid targets acetylcholine receptors (Abbink 1991, Hosie et al. 1995). This difference led me to initially hypothesize that there was a synergistic effect between them. The experiment in chapter II showed that fipronil increased the toxicity of imidacloprid while imidacloprid reduced the toxicity of fipronil. The objective of this chapter was to validate and extend the results of chapter II. Bioassays evaluating the mortality of Formosan subterranean termites exposed to gradient concentration combinations of fipronil and imidacloprid and low-concentration combinations of fipronil and imidacloprid were conducted. In addition, in order to explore the relationship between behavior and mortality, excavation holes, defined as discrete cavities in the sand along the inner margin of the Petri dish, were recorded in the low-concentration combination bioassay.

Materials and Methods

Termites. Formosan subterranean termites, *C. formosanus* were collected using a crate-trapping technique (Smith et al. 2004, Gautam and Henderson 2010) in New Orleans, Louisiana in 2008. The crate trap was kept in a 140-L trash can with a lid and was stored in the urban entomology laboratory under constant conditions (26 - 28°C, 70-80% RH). Prior to the start of the trials, healthy and active workers and soldiers were transferred from a crate trap (one colony)

to a plastic container with moist brown paper towels (Tork Universal, SCA Tissue North America, Neenah, WI).

Termiticides. The two termiticides tested in the trials were fipronil (Fipronil Tech., BAS 350I, BASF Corporation, Research Triangle Park, NC) and imidacloprid (Premise 75, Bayer Corporation, Kansas City, MO).

Gradient combinations bioassay. Fifty grams of autoclaved dry sand (construction sand, Louisiana Cement Products, LLC., Baton Rouge, LA) were loaded in each Petri dish (100 mm × 15 mm, Medegan Medical Products, Gallaway, TN) and a filter paper (55mm in diameter, Grade 1 and Grade 2, Whatman) was placed on top of the sand. Termiticide solutions were prepared by dissolving pre-weighed termiticides with water in 100 ml volumetric flasks. The termiticide weight, concentrations in the solution and concentrations in the sand and Petri dishes in each treatment are shown in Table 3.1. In order to ensure fipronil was mixed with water completely (no visible particles), the volumetric flasks were placed in an ultrasonic bath (Bransonic 1510R-MT ultrasonic cleaner, Danbury, CT) working at 70 W and 42 kHz for 20 - 30 minutes. A 10 ml solution from each termiticide formulation was added to the filter paper in the Petri dishes and 10 ml water only was added as the control.

Fifty termites (45 workers and 5 soldiers) were introduced from the plastic container aforementioned to each treated Petri dish by a soft fine brush. One colony was used in the

bioassay. Then every Petri dish was sealed with Parafilm® (Parafilm M, Pechiney Plastic Packaging, Chicago, IL) and stored in an isolated chamber at room temperature (21 – 23°C).

Each treatment was tested at three exposure times, 24, 48 and 66 hours and three replicates were performed. Three Petri dishes in each treatment were randomly taken out of the chamber at each time period. The number of surviving termites, defined as any part of the termite moving with or without the stimulus from a brush was recorded.

Low-concentration combination bioassays. Fifty grams of autoclaved dry sand was loaded in every Petri dish (100 mm × 15 mm, Medegan Medical Products, Gallaway, TN) and a filter paper (55mm in diameter, Grade 1 and Grade 2, Whatman) were placed on top of the sand. Termiticide solutions were prepared by dissolving pre-weighed termiticides with water in 100 ml volumetric flasks. The termiticide weight, concentrations in the solution and concentrations in the sand and Petri dishes in each treatment are shown in Table 3.2. A 10 ml solution from each termiticide formulation was added to the filter paper in the Petri dishes and 10 ml water only was added as the control.

Fifty termite workers were introduced from the plastic container aforementioned (a different colony from the colony in gradient combination bioassay) to each treated Petri dish by a soft fine brush. Every Petri dish was then sealed with Parafilm® (Parafilm M, Pechiney Plastic Packaging, Chicago, IL) and stored in an isolated chamber at room temperature (21 – 23°C).

Table 3.1. List of treatments and their concentrations in solutions and substrates in gradient combination bioassays.

Treatments	Insecticide weight (mg)	Concentrations in solutions (ppm)	Concentrations in sand and filter paper (ppm)
100 ppm F*	10.48 (F)	≈ 100 (F)	16.68 (F)
80 ppm F + 20 ppm I*	8.43 (F) + 2.67 (I)	≈ 80 (F) + 20 (I)	13.42 (F) + 3.34 (I)
60 ppm F + 40 ppm I	6.16 (F) + 5.28 (I)	≈ 60 (F) + 40 (I)	9.80 (F) + 6.60 (I)
40 ppm F + 60 ppm I	4.30 (F) + 7.93 (I)	≈ 40 (F) + 60 (I)	6.84 (F) + 9.91 (I)
20 ppm F + 80 ppm I	2.17 (F) + 10.65 (I)	≈ 20 (F) + 80 (I)	3.45 (F) + 13.31 (I)
100 ppm I	13.41 (I)	≈ 100 (I)	16.76 (I)
Control	0	0	0

*F: fipronil; I: imidacloprid

Table 3.2. List of treatments and their concentrations in solutions and substrates in the low-concentration bioassays.

Treatments	Insecticide weight (mg)	Concentrations in solutions (ppm)	Concentrations in sand and filter paper (ppm)
10 ppm F*	1.05 (F)	≈ 10 (F)	1.67 (F)
10 ppm F + 10 ppm I*	1.05 (F) + 1.33 (I)	≈ 10 (F) + 10 (I)	1.67 (F) + 1.66 (I)
5 ppm F + 5 ppm I	0.52 (F) + 0.67 (I)	≈ 5 (F) + 5 (I)	0.83 (F) + 0.83 (I)
10 ppm I	1.33 (I)	≈ 10 (I)	1.66 (I)
Control	0	0	0

*F: fipronil; I: imidacloprid

Each treatment was tested at three exposure times, 72, 96 and 120 hours and three replicates were performed. Three Petri dishes in each treatment were randomly taken out of the chamber at each time period. The number of surviving termites, defined as any part of the termite moving with or without the stimulus from a brush was recorded. In addition, the number of excavation holes, defined as discrete cavities in the sand along the inner margin of the Petri dish was recorded (Fig. 3.1).

After recording survivorship in each Petri dish, surviving termites were transferred using clean forceps to untreated Petri dishes (100 mm × 15 mm, Medegan Medical Products, Gallaway, TN) which were pre-loaded with 50 g autoclaved dry sand, a filter paper (55mm in diameter, Grade 1 and Grade 2, Whatman) and 10 ml water. All untreated Petri dishes were labeled with the previous Petri dish treatments and exposure time (72, 96 and 120 hrs) and were stored in the dark drawer at room temperature (21 – 23°C). Surviving termites were counted again in Petri dishes after 44 hours.

Data analysis. A two-way ANOVA was used to analyze the variance of surviving numbers between different treatments at each time and each treatment among different times by using a generalized linear model (PROC GLM) in SAS software (SAS 9.1, SAS Institute, NC). The means of survival numbers were separated using a Least Significant Difference test (LSD) ($\alpha=0.05$).



Fig. 3.1. A Petri dish with excavation holes in the low-concentration bioassay.

The number of excavation holes between different times and between different treatments was analyzed by a two-way ANOVA. The means of hole numbers were separated by a Least Significant Difference test (LSD) ($\alpha=0.05$). A Simple Linear Regression (SLR) was used to analyze the relationship between the mortality and the number of excavation holes for different termiticide treatments at each time period.

In the transfer study of low-concentration bioassay, the percentage of mortality = dead termite numbers/ total termite numbers transferred.

Results

Gradient combination bioassay. There was a significant interaction between treatment and time ($F = 6.05$; $df = 12, 62$; $P < 0.0001$). A significant difference in survival numbers was found among the treatments tested at each time period ($F = 21.00$; $df = 6, 62$; $P < 0.0001$) (Figure 3.2). The survival numbers in the treatment of 80 ppm fipronil plus 20 ppm imidacloprid were significantly higher than in the treatment of 100 ppm fipronil alone at 48 and 66 hours but not at 24 hours (Figure 3.2). Among the combination treatments, the survival numbers in 40 ppm fipronil plus 60 ppm imidacloprid were lowest but still higher than in fipronil alone at 48 and 66 hours (Figure 3.2).

When the data were analyzed within each treatment among different times, a significant difference in survival numbers was found ($F = 39.59$; $df = 2, 62$; $P < 0.0001$) (Fig. 3.3). The survival numbers were significantly reduced in the treatment of 100 ppm fipronil and treatment of 20 ppm fipronil plus 80 ppm imidacloprid at all time intervals (Fig. 3.3). The changes of survival numbers in the treatment of 100 ppm imidacloprid and treatment of 80 ppm fipronil plus 20 ppm imidacloprid among different times were not significantly different (100 ppm imidacloprid: $F = 1.02$; $df = 2, 8$; $P = 0.4163$; 80 ppm fipronil plus 20 ppm imidacloprid: $F = 0.38$; $df = 2, 8$; $P = 0.6994$) (Fig. 3.3). There was no significant difference in survival numbers in the controls ($F = 0.86$; $df = 2, 8$; $P = 0.4705$).

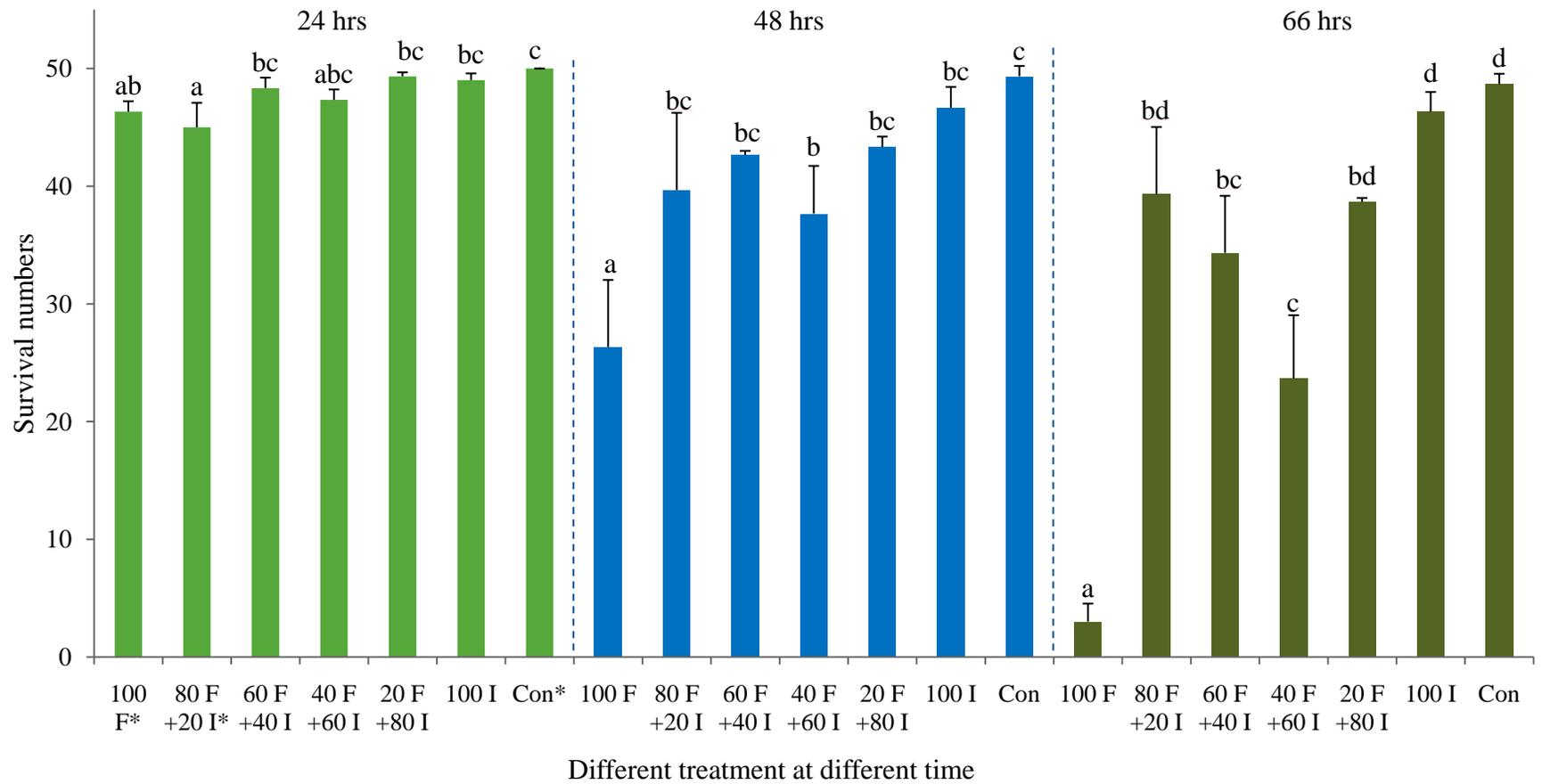


Fig. 3.2. Survival numbers in different treatments at different times. Means with the same letter are not significantly different.

*F: fipronil; I: imidacloprid; Con: control.

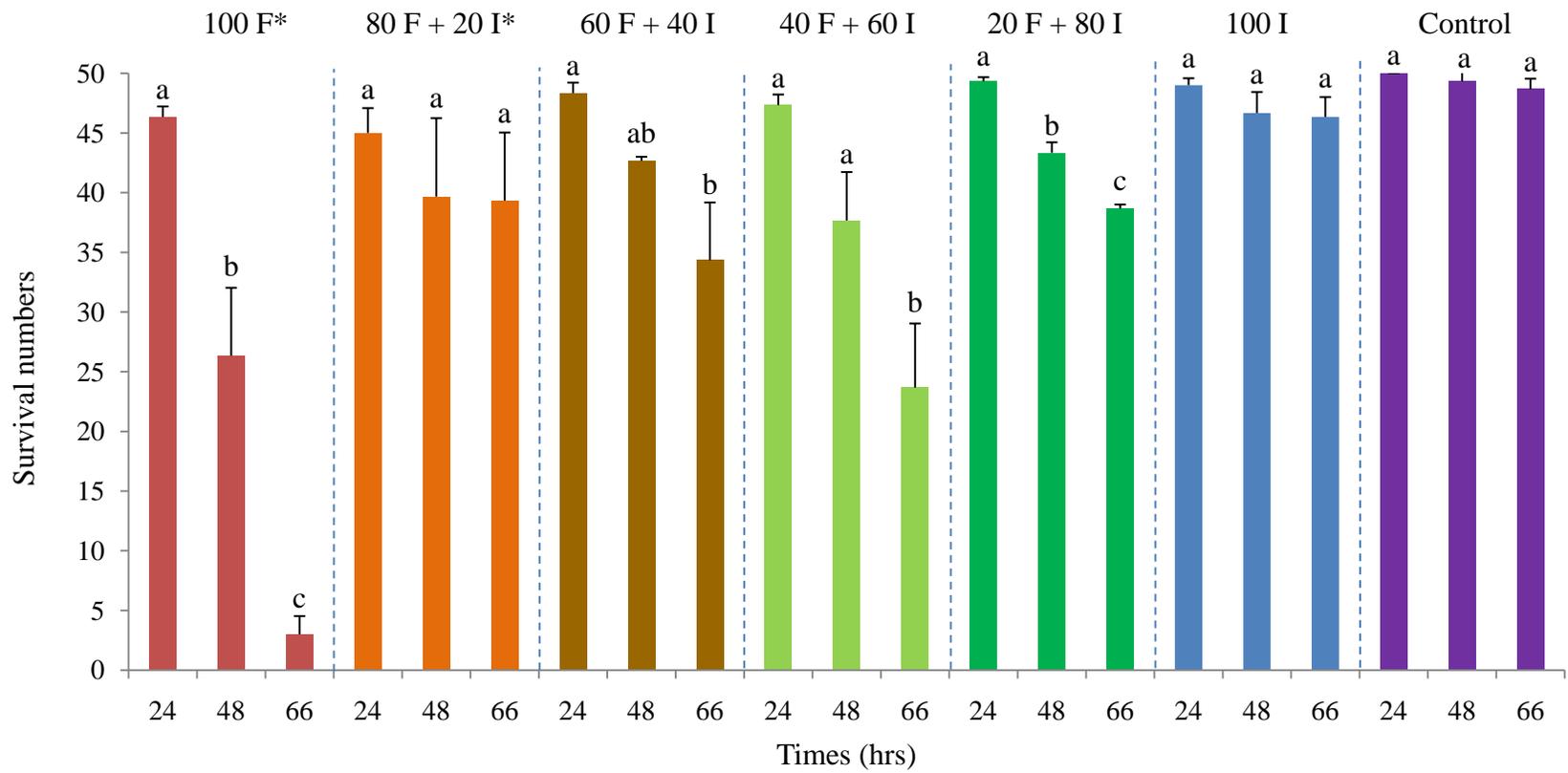


Fig. 3.3. Survival numbers in each treatment at different times. Means with the same letter are not significantly different.
 * F: fipronil; I: imidacloprid.

Low-concentration bioassay. There was a significant interaction between treatment and time ($F = 3.22$; $df = 8, 44$; $P = 0.009$). A significant difference in survival numbers was found among the treatments at each time period ($F = 23.41$; $df = 4, 44$; $P < 0.0001$) (Figure 3.4). The survival numbers in the treatment of 5 ppm fipronil plus 5 ppm imidacloprid were significantly higher than in the treatment of 10 ppm fipronil plus 10 ppm imidacloprid but lower than 10 ppm imidacloprid at 96 hours (Figure 3.4). Among all termiticide treatments at 120 hours, the survival numbers in the treatment of 5 ppm fipronil plus 5 ppm imidacloprid were significantly higher than other termiticide treatments. There was no significant difference between treatments of 10 ppm fipronil and 10 ppm fipronil plus 10 ppm imidacloprid at all time intervals. No significant difference was found between termiticide treatments at 72 hours. One of the Petri dishes treated with imidacloprid alone was heavily infested by fungi (Figure 3.4).

When the data were analyzed within each treatment among different times, a significant difference in survival numbers was found ($F = 9.61$; $df = 2, 44$; $P = 0.0006$) (Fig. 3.5). The survival numbers in the treatment of 10 ppm fipronil dropped to less than 10% at 72 hours and 100% mortality was approached at 96 and 120 hours. In contrast, the survival number in treatment of 10 ppm imidacloprid was much higher at 72 and 96 hours and were not significantly decreased until 120 hours. There was no significant difference in the combination of 5 ppm fipronil plus 5 ppm imidacloprid among all time intervals ($F = 1.86$; $df = 2, 8$; $P = 0.2351$); while

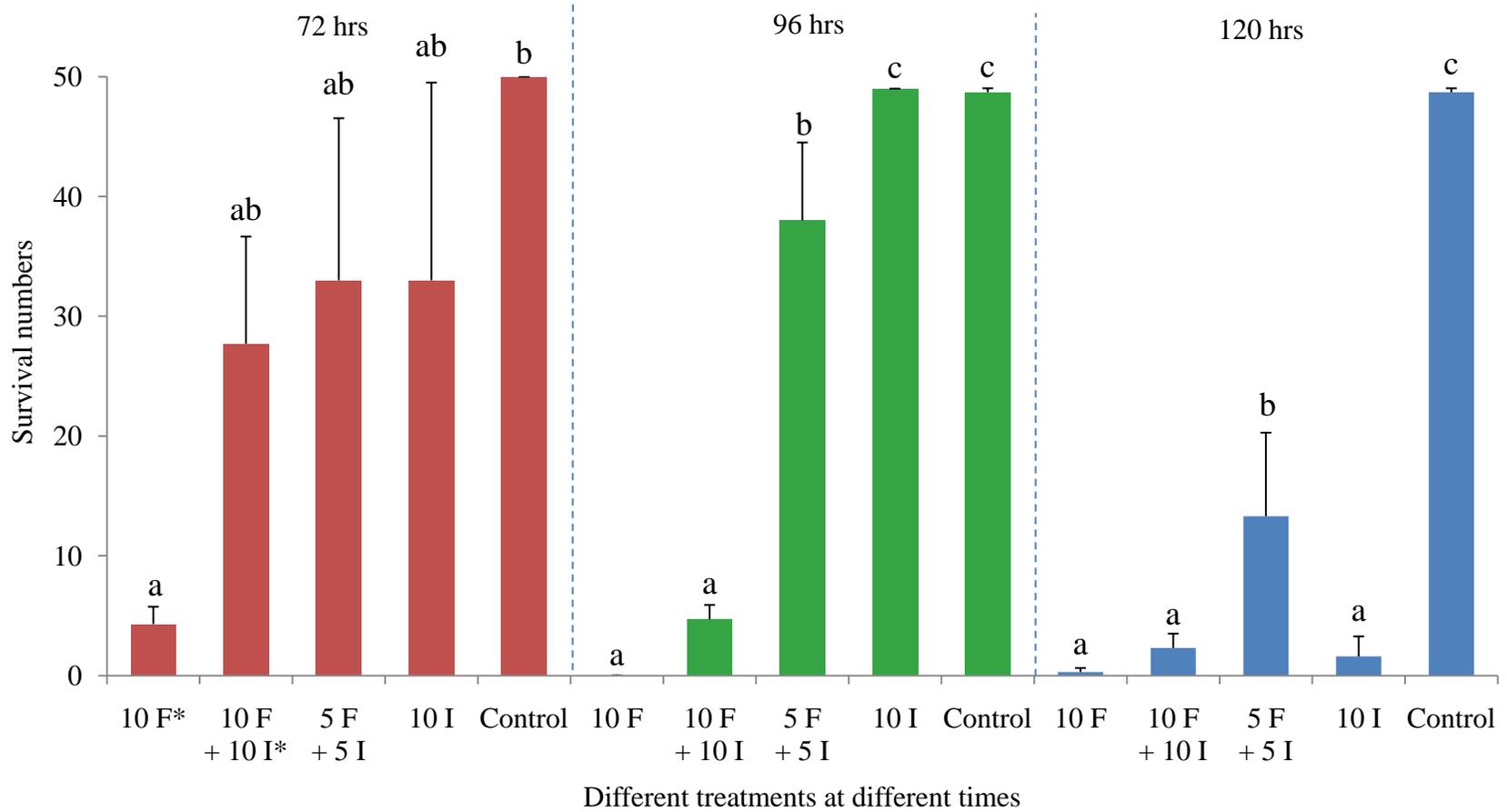


Fig. 3.4. Survival numbers in different treatments at each time. Means with the same letter are not significantly different.

* F: fipronil; I: imidacloprid.

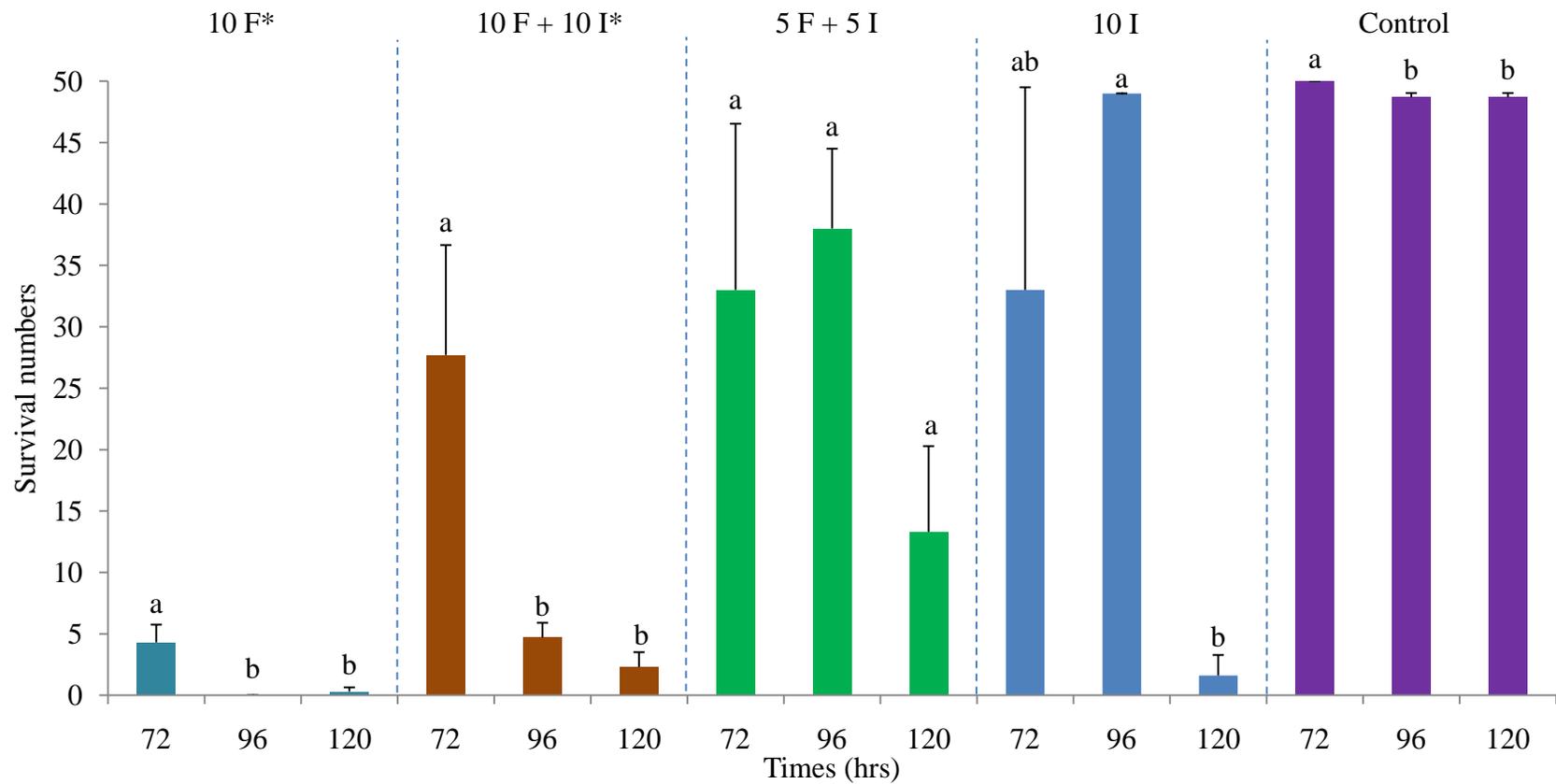


Fig. 3.5. Survival numbers in each treatment at different times. Means with the same letter are not significantly different.

* F: fipronil; I: imidacloprid.

a significant decrease was found at 96 hours in the combination of 10 ppm fipronil plus 10 ppm imidacloprid.

Since there was no significant difference of the number of excavation holes between 72, 96 and 120 hours ($F = 0.76$; $df = 2, 44$; $P = 0.4729$), the number of holes was pooled from different times for the analysis. There was a significant difference in the number of excavation holes among the treatments ($F = 21.46$; $df = 4, 44$; $P < 0.0001$) (Fig. 3.6). The number of excavation holes in treatments of 10 ppm fipronil and 5 ppm fipronil plus 5 ppm imidacloprid was significantly higher than in the control. In contrast, the number of excavation holes in treatments of 10 ppm fipronil plus 10 ppm imidacloprid was significantly lower than in the control (Fig. 3.6). The number of excavation holes was not significantly related with mortality (72 hours: $R^2 = 0.0747$, $P = 0.3901$; 96 hours: $R^2 = 0.0120$, $P = 0.7348$; 120 hours: $R^2 = 0.0867$, $P = 0.3530$).

After surviving termites were transferred to untreated Petri dishes, the mortality of termites previously treated with 10 ppm fipronil plus 10 ppm imidacloprid and 5 ppm fipronil plus 5 ppm imidacloprid for 72 and 96 hours were higher than in previous treatments of 10 ppm imidacloprid and control (Table 3.3).

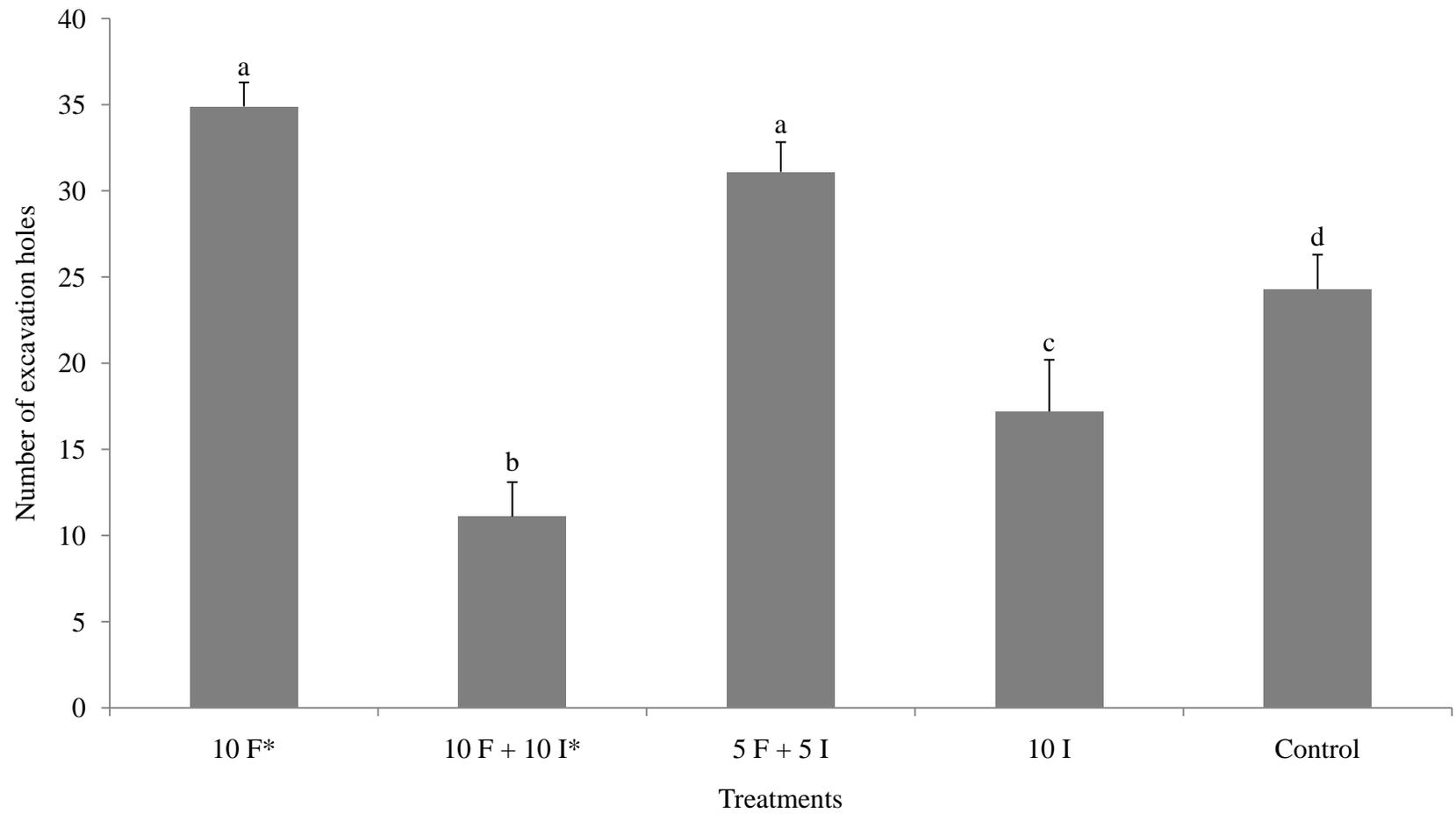


Fig. 3.6. Excavation holes in different treatments. Means with the same letter are not significantly different.

* F: fipronil; I: imidacloprid.

Table 3.3. Percentage of mortality of termites in untreated Petri dishes.

Exposure time in the previous treatment (hrs)	Previous treatments	Percentage of mortality (mean \pm SD) (%)
72	Control	4 \pm 0
	10 ppm F*	100 \pm 0
	10 ppm F + 10 ppm I*	72.16 \pm 12.84
	5 ppm F + 5 ppm I	78.15 \pm 25.59
	10 ppm I	2.02 \pm 0.03
96	Control	8.19 \pm 4.04
	10 ppm F	N/A*
	10 ppm F + 10 ppm I	79.37 \pm 18.03
	5 ppm F + 5 ppm I	84.29 \pm 13.95
	10 ppm I	6.80 \pm 2.36
120	Control	5.48 \pm 3.13
	10 ppm F	N/A
	10 ppm F + 10 ppm I	100 \pm 0
	5 ppm F + 5 ppm I	91.36 \pm 7.71
	10 ppm I	N/A

* F: fipronil; I: imidacloprid. N/A: not available. No termites were transferred to untreated Petri dishes due to no surviving termites were found in the previously treated Petri dishes.

Discussion

The results in the gradient combination bioassay supported the hypothesis that imidacloprid reduced the efficacy of fipronil. The result showed that a small amount of imidacloprid (20 ppm in the solution = 3.34 ppm in the substrates) despite a relatively high concentration of fipronil led to a sharp increase in survival numbers (Fig. 3.1).

The result that survivorship in all treatments at 24 hours was higher than 90% can be explained by the delayed toxicity of fipronil and imidacloprid. The delayed feature of fipronil was reported in bioassays in Western subterranean termites, Eastern subterranean termites and Formosan subterranean termites where a delay of around three days in low concentrations of fipronil was observed (Ibrahim et al. 2003, Remmen and Su 2005, Saran and Rust 2007). For imidacloprid, the delayed toxicity was corroborated by the findings of Haagsma (2003) and Ramakrishnan et al. (2000) and was also noted in chapter II. It was surprising that the survivorship in treatments of four combinations and imidacloprid alone showed a “V” shape at 48 and 66 hours: the survivorship in the treatment of 40 ppm fipronil plus 60 ppm imidacloprid was lower than treatments of 60 ppm fipronil plus 40 ppm imidacloprid, 20 ppm fipronil plus 80 ppm imidacloprid and imidacloprid alone. The increasing trend in treatments of 40 ppm fipronil plus 60 ppm imidacloprid, 20 ppm fipronil plus 80 ppm imidacloprid and imidacloprid alone was due to the decrease of the fipronil concentration. However, it is unknown why there was a decreasing trend in treatments of 80 ppm fipronil plus 20 ppm imidacloprid, 60 ppm fipronil plus 40 ppm imidacloprid.

The changes of survivorship in the low-concentration bioassay were not as distinct as in gradient combination bioassay. Firstly, no significant difference was found between termiticide combinations and control at 72 hours (Fig. 3.4). This result was most likely caused by a large

variation in the limited numbers of replicates. For example, one of three replicates in the treatment of 10 ppm imidacloprid alone was heavily infested by fungi and the survival number was zero, while the mean of survival numbers in the other two dishes dropped only 1%.

Secondly, the survivorship at 96 hours showed an increasing trend with the decreased concentration of fipronil. This relationship was due to the delayed toxicity of imidacloprid in the bioassay: mortality was only determined by fipronil. At 120 hours, interestingly, the mortality in the treatment of 5 ppm fipronil plus 5 ppm imidacloprid was significantly lower than treatment of 10 ppm fipronil alone and 10 ppm imidacloprid alone, which suggests an antagonistic effect between low concentrations of fipronil and imidacloprid.

The lower number of excavation holes in dishes with imidacloprid demonstrated that imidacloprid impaired digging behavior (Fig. 3.6). This result was consistent with findings in other studies which noted imidacloprid inhibited termite digging and tunneling behavior (Thorne and Breisch 2001, Henderson 2003). However, mortality was not significantly related with excavation holes in the bioassay. This may be due to the lag time between the effect of behavior inhibition and lethal effect. Remmen and Su (2005) reported low concentrations of imidacloprid would not kill termites immediately but the effect of behavior inhibition showed up relatively early (Thorne and Breisch 2001, Henderson 2003). Therefore, when termites stopped digging excavation holes, they remained alive.

In the transfer study, the difference in percentages of mortality can also be explained by the variance in toxicity of fipronil and imidacloprid (Table 3.3). According to survivorship changes in Fig. 3.5, imidacloprid did not show significant toxicity until 120 hours while fipronil exhibited toxicity at 72 hours. Therefore, the fipronil residue in the termites' body led to a high mortality in termites previously exposed to treatments with fipronil (fipronil alone and the combinations) between 72 hours and 120 hours. In contrast, due to the delayed toxicity of imidacloprid, the imidacloprid residue did not lead to high mortality before 120 hours and therefore mortality in termites previously treated with imidacloprid alone was less than 10% at 72 and 96 hours.

Laboratory bioassays are important tools for modeling applications of termiticides in the field. This chapter attempted to more precisely discover the toxicity interaction of fipronil and imidacloprid. Since both fipronil (Termidor®) and imidacloprid (Premise®) have been widely used in the termite control in the United States, it is quite possible that perimeters of houses may be treated with both of them. The laboratory results suggest that the efficacy of Termidor® may be negatively affected by Premise® in the field. The actual concentration of imidacloprid in the soil after treatment is believed to be around 50 ppm and the half-life of imidacloprid is reported in the range from 990 to 1,230 days (Baskaran et al. 1999). Therefore the concentration of Premise® residue could stay high for a long time and the efficacy of Termidor® could be

reduced during this period. However, the field efficacy of termiticides is not determined only by their toxicity but also by their bioavailability, soil properties and microbial degradation (Ramakrishnan et al. 2000, Osbrink et al. 2005, Tsunoda 2006). The evaluation of interaction of Termidor® and Premise® in the field would be necessary.

Although the result that low concentrations of imidacloprid in the combination led to a significant increase in survivorship than fipronil alone, it was still challenged by another factor—the changes of fipronil concentrations. It is unknown that whether this decrease in mortality was caused by the decrease of fipronil. An experiment with constant concentration of fipronil and the variable in concentration of imidacloprid should be conducted to further validate the result.

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CHAPTER 4

CHANGES IN SURVIVORSHIP AND BEHAVIOR IN *COPTOTERMES FORMOSANUS* DUE TO GRADIENT CONCENTRATIONS OF IMIDACLOPRID COMBINED WITH A FIXED AMOUNT OF FIPRONIL

Introduction

Behavior studies provide important clues to understanding conundrums in eusocial insects such as termites (Whitman and Forschler 2007). Some behaviors such as trophallaxis, grooming, cannibalism and coprophagy enable the spread of toxicant between individuals in a colony. However it was reported that physical contact was the main pathway of uptake and transfer of fipronil and imidacloprid (Haagsma 2003, Saran and Rust 2007, Bagnères et al. 2009). Metabolism studies of imidacloprid in *Reticulitermes flavipes* (Kollar) showed that the metabolites such as olefin-imidacloprid were less toxic than imidacloprid itself (Tomalski et al. 2010). Due to hyperexcitation of the nervous system imidacloprid inhibited walking, digging and tunneling behavior in termites (Thorne and Breisch 2001, Henderson 2003).

In the bioassay results of this chapter and the low-concentration combination bioassay in chapter III, excavation holes were used as an indication of behavioral effects. The literature has described excavation behavior infrequently. Whitman and Forschler (2007) divided the whole process of construction excavation paths into four phases: pill (shaped substrates by mouthpart) formation, pill transportation, pill deposition and return to the excavation site. It is reported that the excavation path is constructed by more than one termite in the first 24 hours and 50% of the time excavation involves contact with the substrate which exposes termites to the toxicant orally (Whitman and Forschler 2007).

Chapter II showed that toxicity interactions were evident between fipronil and imidacloprid. Chapter III revealed that low concentrations of imidacloprid could significantly reduce the efficacy of fipronil. However, there were two variables (increasing imidacloprid and decreasing fipronil) that influenced the survivorship of termites. As such I hypothesized that there was a threshold concentration of imidacloprid that would significantly reduce the toxicity of fipronil. In addition, since imidacloprid has significant effects on termite behavior, I hypothesized that there was a relationship between unimpaired behavior such that termites would remain active similar to controls and mortality. The objective of this chapter was to further explore the toxicity interaction between fipronil and imidacloprid and investigate the mechanism behind it by conducting a bioassay having a constant concentration of fipronil and a gradient of imidacloprid concentrations.

Materials and Methods

Termites. Formosan subterranean termites, *C. formosanus* were collected using a crate-trapping technique (Smith et al. 2004, Gautam and Henderson 2010) in New Orleans, Louisiana in 2008. The crate trap was kept in a 140-L trash can with a lid and was stored in the urban entomology laboratory under constant conditions (26 - 28°C, 70-80% RH). Prior to the start of the trials, healthy and active workers and soldiers were transferred from the crate trap (one

colony) to a plastic container with moist brown paper towels (Tork Universal hand towel, SCA Tissue North America, Neenah, WI).

Termiticides. The two termiticides tested in the trials were fipronil (Fipronil Tech., BAS 350I, BASF Corporation, Research Triangle Park, NC) and imidacloprid (Premise 75, Bayer Corporation, Kansas City, MO).

Bioassays. Seventy-five grams of autoclaved dry sand (construction sand, Louisiana Cement Products, LLC., Baton Rouge, LA) were loaded in each Petri dish (100 mm × 15 mm, Medegan Medical Products, Gallaway, TN) and a filter paper (55mm in diameter, Grade 1 and Grade 2, Whatman) was placed on top of the sand. Termiticide solutions were prepared by dissolving pre-weighed compounds with water in 100 ml volumetric flasks. In order to ensure fipronil was mixed in water completely (no visible particles), the volumetric flasks were placed in an ultrasonic bath (Bransonic 1510R-MT ultrasonic cleaner, Danbury, CT) working at 70 W and 42 kHz for 20 - 30 minutes. The five termiticide solutions prepared for the bioassay and the concentrations of each termiticide in the substrate are shown in Table 4.1. A 15 ml solution from each termiticide formulation was added to the filter paper in the Petri dishes and 15 ml water only was added as the control.

Fifty termites, (45 workers and 5 soldiers) were introduced from the plastic container aforementioned to each treated Petri dish by a soft fine brush. Then every Petri dish was sealed

Table 4.1. List of treatments and their concentrations in solutions and substrates.

Treatments	Concentrations in solutions (ppm)	Concentrations in substrates (ppm)
100 ppm F*	100 F	18.82 F
100 ppm F + 5 ppm I*	100 F + 5 I	18.82 F + 0.79 I
100 ppm F + 15 ppm I	100 F + 15 I	18.82 F + 2.29 I
100 ppm F + 25 ppm I	100 F + 25 I	18.82 F + 3.79 I
100 ppm I	90 I	15.08 I
Control	0	0

*F: fipronil; I: imidacloprid.

with Parafilm® (Parafilm M, Pechiney Plastic Packaging, Chicago, IL) and stored in an isolated chamber at room temperature (21 – 23°C).

Each treatment was tested at 48 and 66 hours and three replicates were performed. Three Petri dishes in each treatment were randomly taken out of the chamber at each time period. The number of surviving termites, defined as any part of the termite moved with or without the stimulus from a brush was recorded. In addition, the number of excavation holes, defined as discrete holes along the inner margin of the Petri dishes was counted.

Data analysis. A two-way ANOVA was used to analyze the variance of survival numbers among different combinations and different times by using a generalized linear model (PROC GLM) in SAS software (SAS 9.1, SAS Institute, NC). The means of survival numbers were separated using a Least Significant Difference test (LSD) ($\alpha=0.05$).

The number of excavation holes between different times and between different treatments was analyzed by a two-way ANOVA. The means of hole numbers were separated by a Least

Significant Difference test (LSD) ($\alpha=0.05$). A Simple Linear Regression (SLR) was used to analyze the relationship between changes in the mortality percentage and changes in number of excavation holes posttreatment at 48 and 66 hours.

Results

The results provided a threshold concentration of imidacloprid that significantly reduces the toxicity of fipronil. Even 2.3 ppm (15 ppm in the solution) imidacloprid reduced the efficacy of fipronil in sand (Fig. 4.1). There was a significant difference between treatments at 48 and 66 hours ($F = 82.53$; $df = 5, 35$; $P < 0.0001$) (Fig. 4.1). The survival numbers in the treatment of 100 ppm fipronil plus 15 ppm imidacloprid were significantly higher than in the treatment with lower imidacloprid concentrations (100 ppm fipronil plus 5 ppm imidacloprid and fipronil alone) at 48 hours (Fig. 4.1). At 66 hours, survival numbers in the treatment of 100 ppm fipronil plus 25 imidacloprid were significantly higher than treatments with lower concentrations of imidacloprid. Therefore the threshold concentration was between 15 ppm and 25 ppm (2.29 ppm and 3.79 ppm in the substrate).

There also was a significant interaction between treatment and time ($F = 9.51$; $df = 5, 35$; $P < 0.0001$). A significant difference in survival numbers was found within each treatment among different times ($F = 73.27$; $df = 1, 35$; $P < 0.0001$) (Fig. 4.2). Survival numbers in treatments of combinations were significantly reduced at 66 hours (Fig. 4.2). The changes of survival numbers

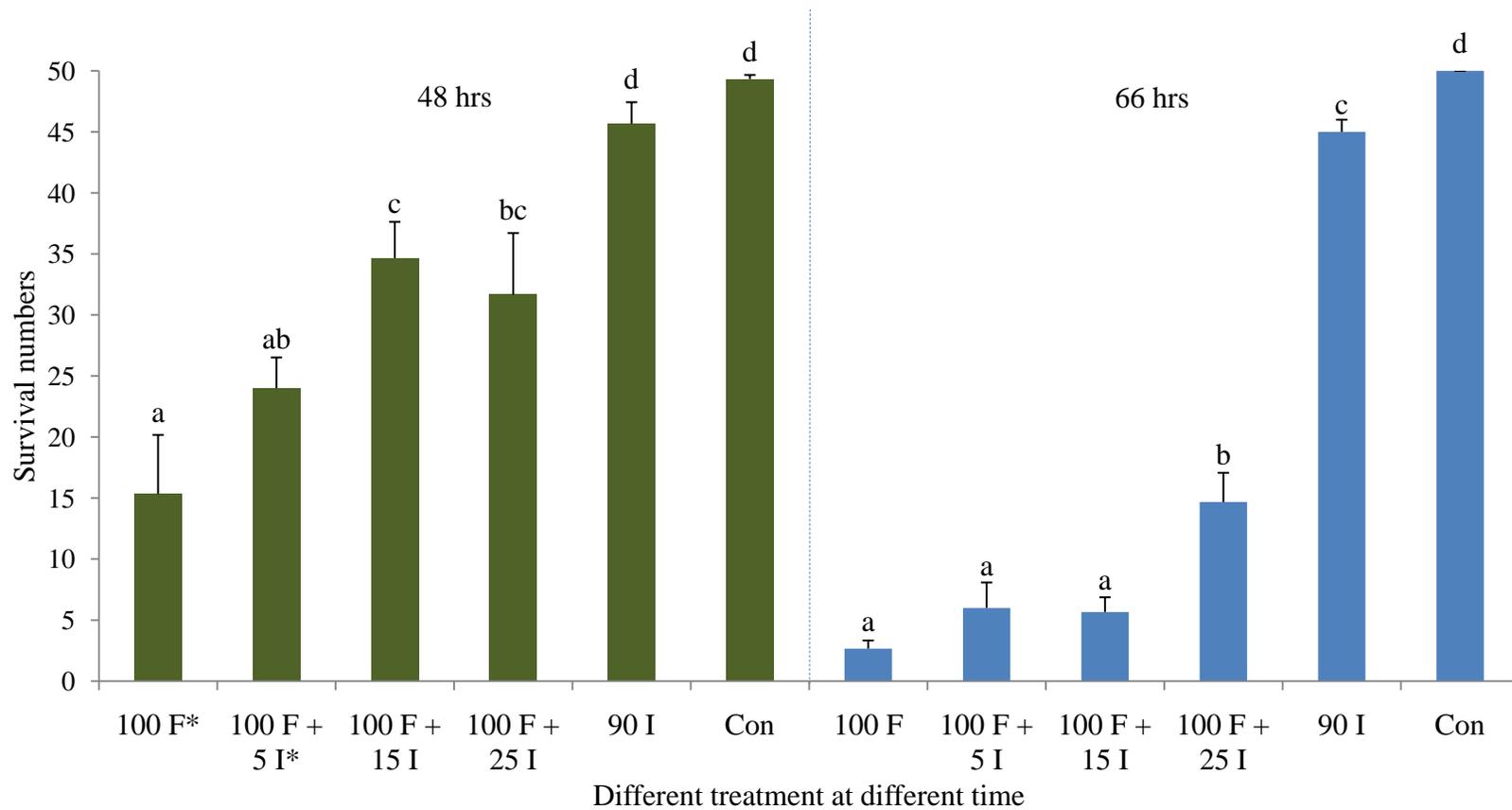


Fig. 4.1. Survival numbers in different treatments at different times. Means with the same letter are not significantly different.
 * F: fipronil; I: imidacloprid; Con: control.

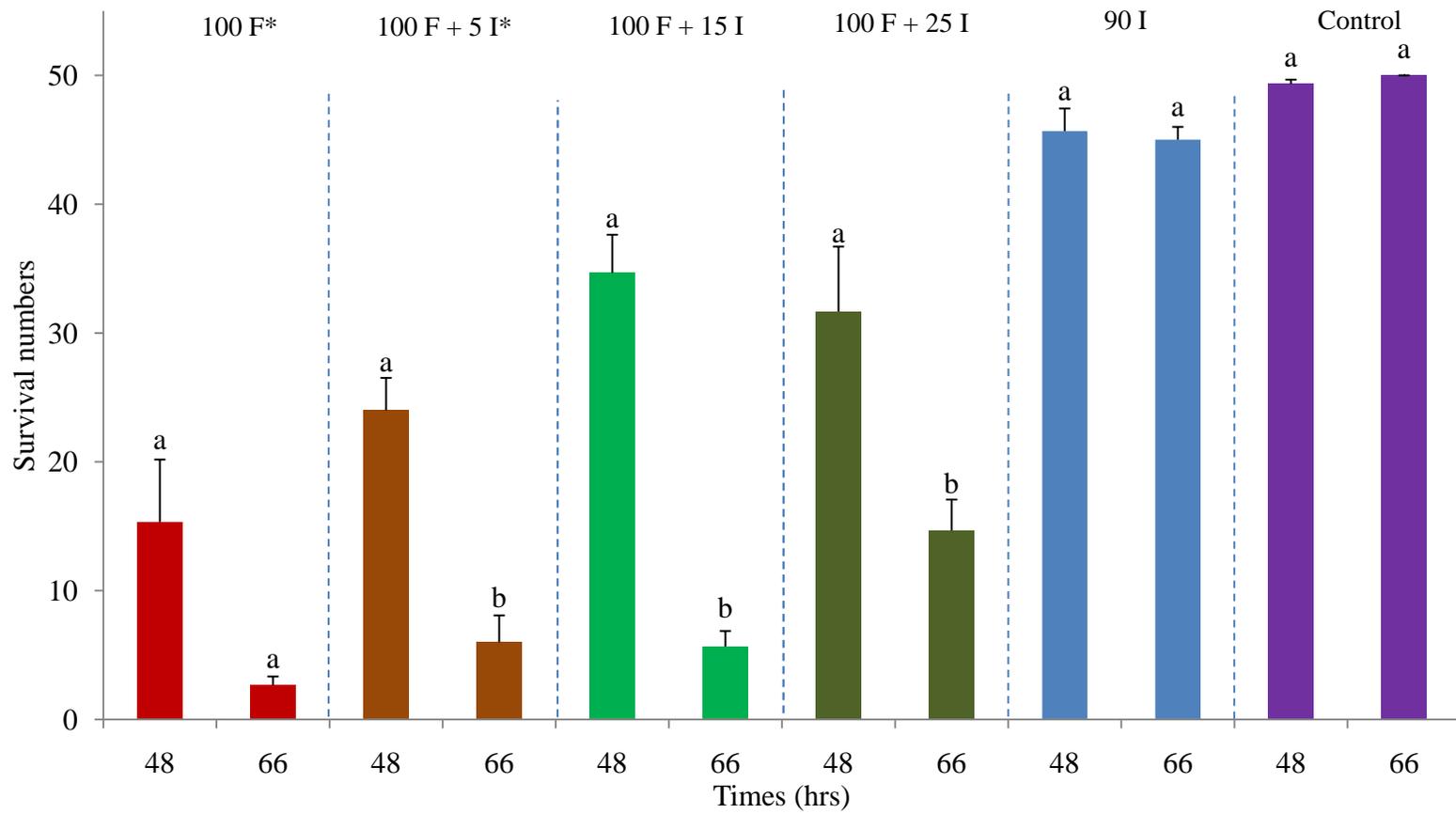


Fig. 4.2. Survival numbers in each treatment at different times. Means with the same letter are not significantly different.
 * F: fipronil; I: imidacloprid.

in the treatment of 90 ppm imidacloprid and 100 ppm fipronil among different times were not significantly different ($F = 0.11$; $df = 1, 5$; $P = 0.7588$; $F = 6.72$; $df = 1, 5$; $P = 0.0606$) (Fig. 4.2). There was no significant difference in survival numbers between time intervals in the controls ($F = 4.00$; $df = 1, 5$; $P = 0.1161$).

Since there was no significant difference in the number of excavation holes between 48 and 66 hours ($F = 0.31$; $df = 1, 35$; $P = 0.5816$), the number of holes was pooled from different different times for the analysis. There was a significant difference in the number of excavation holes among the treatments ($F = 31.92$; $df = 5, 35$; $P < 0.0001$). The number of excavation holes was significantly higher in all the combinations than in imidacloprid alone but lower than in fipronil alone (Fig. 4.3) The number of excavation holes was significantly related to termite mortality at 48 and 66 hours (48 hours: $R^2 = 0.6588$, $P = 0.0002$; 66 hours: $R^2 = 0.4291$, $P = 0.008$) (Fig. 4.4).

Discussion

The discovery of the threshold of imidacloprid interaction with fipronil provides a precise description of pharmacodynamic patterns. Excavation holes showed a decrease as concentrations of imidacloprid increased (Fig. 4.3). In addition, the significant relationship between numbers of excavation holes and mortality provided an important clue to interpret this discovery (Fig. 4.5). Whitman and Forschler (2007) reported excavation building exposed termites to an oral dose of

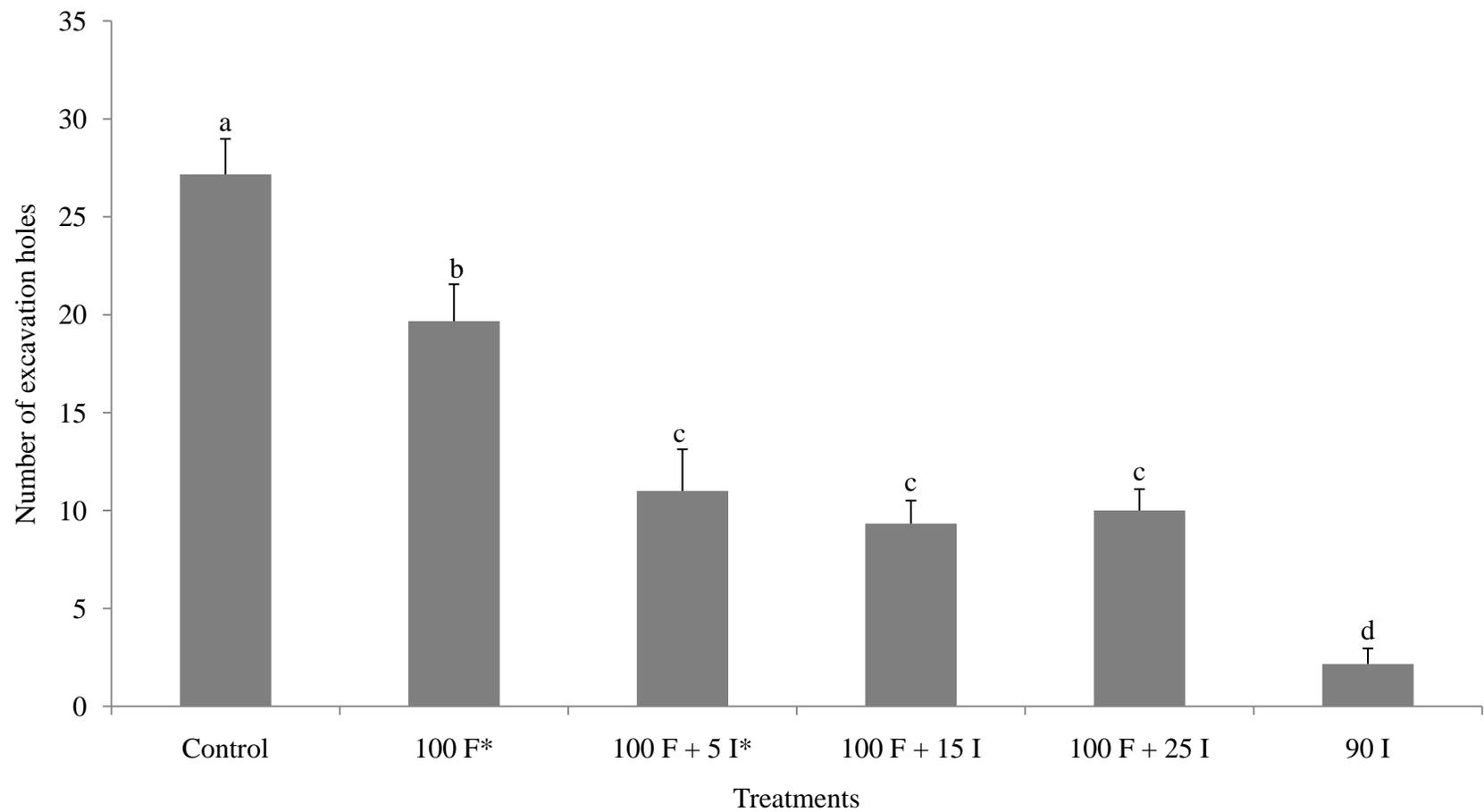


Fig. 4.3. Excavation holes in different treatments. Means with the same letter are not significantly different.
* F: fipronil; I: imidacloprid.

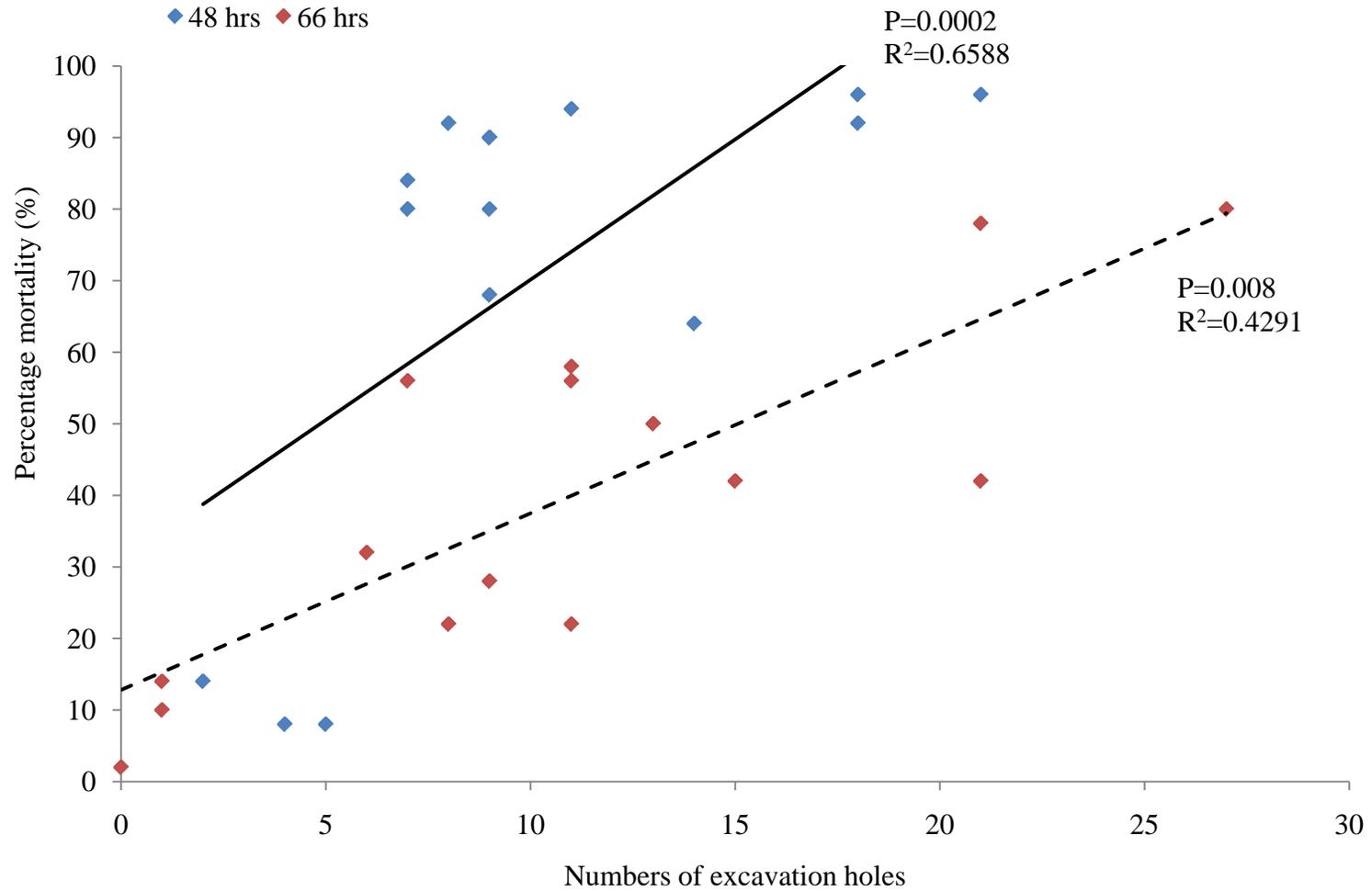


Fig. 4.4. The relationship of changes in numbers of excavation holes and changes in percentage mortality of termites.

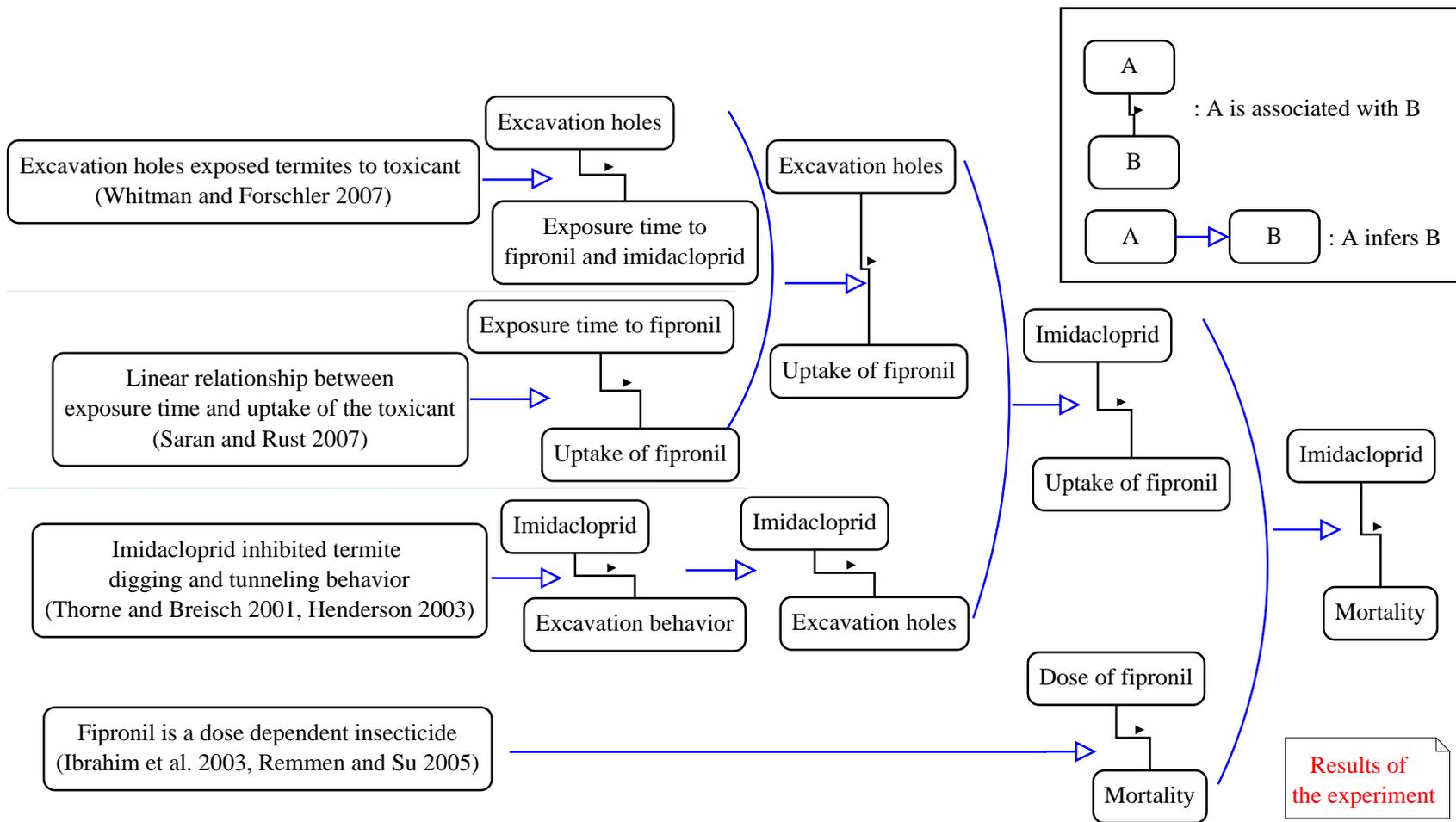


Fig. 4.5. The mechanism of imidacloprid leading to a lower mortality in the combinations than in fipronil alone.

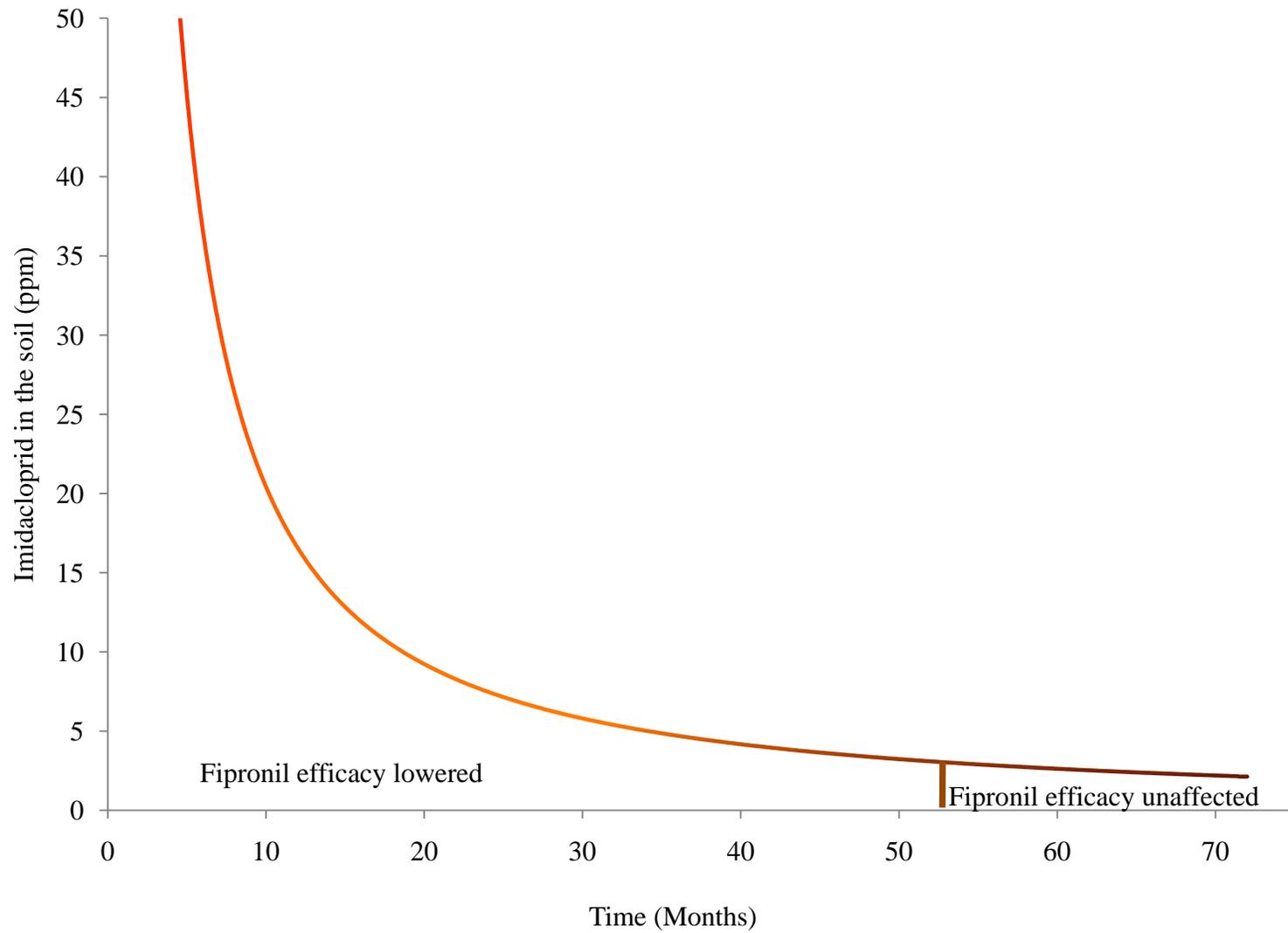


Fig. 4.6. The threshold concentration of imidacloprid that had negative effects on fipronil.

the toxicant. Therefore, excavation holes were positively linked with the exposure time of termiticides and more excavation holes indicated a longer exposure time. In addition, there was a linear relationship between the time of exposure and uptake of the toxicant (Saran and Rust 2007). Thus more excavation holes in a Petri dish treated with combinations of fipronil and imidacloprid were associated with a higher uptake of fipronil (Fig. 4.5). On the other hand, imidacloprid was reported to inhibit termite behavior (Thorne and Breisch 2001; Henderson 2003). Chen and Allen (2006) also reported a relationship between digging behavior in fipronil-treated sand and mortality in the red imported fire ant *Solenopsis invicta* Buren. Thus the presence of imidacloprid led to a smaller number of excavation holes. Since less excavation holes were associated with lower uptake of fipronil, the imidacloprid caused a less uptake of fipronil (Fig. 4.5). Moreover, fipronil is a dose dependent termiticide which means a higher concentration leads to a higher mortality (Ibrahim et al. 2003; Remmen and Su 2005). Therefore imidacloprid is associated with the mortality. It is not surprising that a lower mortality was observed when imidacloprid was present in the combinations (Fig. 4.5).

Saran and Rust (2007) noted that fipronil also inhibited termite tunneling behavior, which seems to challenge the proposed mechanism. However, the inhibition caused by fipronil could only be noticed after the onset of acute toxicity which was around three days posttreatment (Ibrahim et al. 2003, Remmen and Su 2005). In contrast, the symptoms caused by imidacloprid

are much faster but of a chronic nature since recovery is possible. For example, in a preliminary experiment, inhibition of walking behavior caused by imidacloprid was noticed as early as two hours. Henderson (2003) also reported that symptoms such as inhibited digging behavior caused by imidacloprid were exhibited much faster than with fipronil. Therefore the inhibited tunneling behavior caused by fipronil is not against the proposed mechanism provided in Fig. 4.5.

Low mortality of termites in treatments with imidacloprid was due to the delayed toxicity (Fig. 4.2). This result was consistent with findings by Haagsma (2003) in bioassays in Western subterranean termites, *Reticulitermes hesperus* Banks and by Ramakrishnan et al. (2000) in *Reticulitermes flavipes* (Kollar). The results in Chapter III of a significant decrease of survivorship in termites treated with imidacloprid was not shown until at 120 hours also supported the results of delayed toxicity.

From the results I suggest that the efficacy of Termidor® (active ingredient: fipronil) could be reduced by the presence of Premise® (active ingredient: imidacloprid). As two of the most popular termiticides in the market, it is not unusual for pest control companies to use one or both of them along the perimeter of houses during retreatment. The average termiticide concentration in the soil after treatment is believed to be around 50 ppm and imidacloprid persists for a long time in the soil (Baskaran et al. 1999). Therefore, before the concentration of Premise® drops to the threshold (2.29 ppm to 3.79 ppm in the substrate), the efficacy of

Termidor® could be negatively affected (Fig. 4.6). However, the field efficacy of termiticides is affected by multiple factors such as bioavailability and soil properties (organic matter, PH, water content) (Ramakrishnan et al. 2000; Osbrink and Lax 2002; Osbrink et al. 2005). The results in the laboratory bioassays provided a basic model for consideration in the field and direct evaluations of toxicity of termiticides in the field are necessary. Analysis of the pharmacodynamics and degradations of fipronil and imidacloprid in the termite body might provide a comprehensive understanding of the mechanism for the interaction.

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CHAPTER 5

SUMMARY AND CONCLUSIONS

Termites belong to the order Isoptera and are some of the most important structural pests.

The goal of the research was to evaluate the toxicity interaction between fipronil and imidacloprid in the Formosan subterranean termite, *Coptotermes formosanus* Shiraki in the laboratory. I initially hypothesized that there was a synergistic effect between them and conducted an experiment testing the effects of two discrete concentrations (50 ppm and 100 ppm) of fipronil and imidacloprid alone and in two combinations and measured the survivorship of termites. Unexpectedly the result indicated that imidacloprid reduced the efficacy of fipronil and fipronil increased the toxicity of imidacloprid. To validate and extend these results two more bioassays – gradient combination bioassay and low-concentration bioassay were performed. The result of the gradient bioassay indicated that even very low concentrations of imidacloprid significantly reduced the efficacy of even high concentrations of fipronil.

Based on the results from the gradient bioassay, I hypothesized that there was a threshold concentration of imidacloprid which would significantly reduce the efficacy of fipronil. In order to test this, another bioassay composed by a set concentration of fipronil and a gradient of concentrations of imidacloprid was conducted. In addition, behavioral observations of termites were included. We predicted that low mortality was related to the inhibition of excavation behavior caused by imidacloprid since the inhibition reduced the exposure time to the toxicant. The result showed a threshold concentration between 15 and 25 ppm (part per million)

imidacloprid that had significant effects on the efficacy of fipronil really existed. Moreover, termite excavation behavior was found significantly related with the mortality of termites. Therefore, it is believed that imidacloprid reduced efficacy of fipronil by inhibiting termite excavation behavior and exposure to insecticide-laced sand.

Both fipronil (under registered name Termidor®) and imidacloprid (under registered name Premise®) are widely used in the termite control market. The results indicate that the efficacy of Termidor® may be affected by the presence of Premise® in the field.

VITA

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