UTILIZATION AND EVALUATION OF AN INDOXACARB-BASED GRANULAR BAIT (ADVION™) DEVELOPED FOR THE CONTROL OF THE RED IMPORTED FIRE ANT, *Solenopsis invicta* BUREN (HYMENOPTERA: FORMICIDAE)

A Dissertation

by

BARRY D. FURMAN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2006

Major Subject: Entomology

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Approved by:

Chair of Committee,	Roger E. Gold
Committee Members,	Darrell E. Bay
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	Timothy P. Scott
Head of Department,	Kevin M. Heinz

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ABSTRACT

Utilization and Evaluation of an Indoxacarb-based Granular Bait (Advion™) Developed for the Control of the Red Imported Fire Ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). (May 2006)

Barry D. Furman, B.S., Texas A&M University;

M.S., Texas A&M University

Chair of Advisory Committee: Dr. Roger E. Gold

This research evaluated the new red imported fire ant (RIFA) bait Advion™. Advion™ contains the active ingredient indoxacarb which, following ingestion, must be metabolized into an N-decarbomethoxyllated metabolite known as JT333 in order to become acutely toxic. Laboratory experimentation was conducted to determine the most effective chemical form and concentration of indoxacarb, as well as the most appropriate grit size, for use in Advion™. The results indicated that Advion™ containing indoxacarb was more effective than Advion™ containing JT333, that 0.10%, 0.06%, and 0.045% were the most effective concentrations of indoxacarb, and that standard sized grit (~2 mm) was more effective than small sized grit (<1 mm).

Field experimentation was conducted to determine the most effective concentration and quantity of Advion™, as well as the most effective placement of the bait, for obtaining maximum control of RIFA colonies via individual mound treatments. The results indicated that 10 g (2 Tbsp) of 0.045% Advion™ placed around the mound in a circle with a radius of 0.5-3.0 m was the most effective manner in which to treat individual RIFA mounds. Field experimentation was also conducted to compare the effectiveness of label-rate broadcast treatment with Advion™ to the effectiveness of both

label-rate broadcast treatment with Amdro® and pre-baiting broadcast treatment with Advion™. Both pre-baiting broadcast treatment and label-rate broadcast treatment with Advion™ ultimately resulted in 98-99% RIFA colony mortality, which was significantly greater than the 87% colony mortality resulting from broadcast treatment with Amdro®. The 6.2 d LT_{90} for label-rate broadcast treatment with Advion™ was nearly one half that of the LT_{90} for pre-baiting broadcast treatment.

Laboratory experimentation was conducted to determine whether RIFA workers were capable of metabolizing indoxacarb into the toxic metabolite, and the results clearly indicated that they were. Finally, field experimentation was conducted to evaluate the effectiveness of label-rate broadcast treatment with Advion™ at controlling multiple ant species. The results indicated that label-rate broadcast treatment with Advion™ resulted in control of the RIFA and *Pogonomyrmex barbatus* for at least 7 wk, and *Monomorium pharaonis* and *Dorymyrmex pyramicus* for some period of time between 3 and 7 wk.

DEDICATION

This work is dedicated to my family, and to all those who inspire others to achieve positive goals in life.

We don't get to choose the times in which we live; what we do get to choose is what to do with the time which we are given.

J.R.R. Tolkien

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CHAPTER I

INTRODUCTION

Since its accidental introduction into the United States in the 1930's via South American ships arriving and offloading in Mobile, Alabama, the red imported fire ant (RIFA), *Solenopsis invicta* Buren, has been the cause of much concern due primarily to its rapid spread and adverse effects on an extremely wide variety of biota (Helms and Vinson 2001; Vinson 1997; Wojcik et al. 2000). In terms of its rapid spread, the RIFA has managed to expand its range throughout the entire southern and southeastern United States and, likely due to interstate commerce, has now managed to become established in some western states, as well. The RIFA currently infests well over 128 million hectares in 14 states, including North Carolina, South Carolina, Florida, Georgia, Alabama, Tennessee, Mississippi, Louisiana, Arkansas, Oklahoma, Texas, New Mexico, Arizona, and California (Drees and Gold 2003). Unfortunately, the range of the RIFA continues to expand, and it is now predicted that the future range of these ants will include such states as Nevada, Oregon, and Washington in the west, and Virginia, Maryland, and Delaware in the east (Korzukhin et al. 2001).

As far as the effects of the RIFA on other organisms within the United States, these ants have clearly demonstrated the ability to adversely affect a wide variety of invertebrates, vertebrates, plants, and even entire ecosystems. In terms of invertebrates,

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This dissertation follows the style and format of the Journal of Economic Entomology.

the RIFA is known to out compete, displace, and/or kill a variety of arthropods ranging from endangered butterflies to other ant species, spiders, and beneficial dung and carrion-inhabiting beetles (Cook 2001; Forys et al. 2001; Hu and Frank 1996; Porter and Savignano 1990; Stoker et al. 1995). Though the RIFA oftentimes displaces and/or kills pest arthropods such as aphids, various lepidopteran larvae, horn flies, and ticks, unfortunately they also commonly displace and/or kill the natural enemies of pest insects with equal vigor, thus commonly creating new problems of equal or greater import (Fleetwood et al. 1984; Kaplan and Eubanks 2002; Lemke and Kissam 1988; Vinson 1997).

A variety of vertebrates are also adversely affected by the RIFA. In certain instances, as is the case with some rodents and birds, the RIFA can have an adverse affect via direct competition for resources. However, the primary threat that these ants pose to vertebrates revolves around the piperidine toxins that are injected via the RIFA's sting. These toxins possess necrotizing properties and can produce alteration of behavior, injury, allergic responses or even death to such varied vertebrates as birds, cattle, fish, sea turtles, and humans (Allen et al. 1994, 2001; Apperson 1983; Barr and Drees 1995a, 1995b; Caro et al. 1978; Contreras and Labay 1999; Drees 1994; Lockley 1974; Parris et al. 2002; Rhoades et al. 1977; Stafford 1996).

Even many plants are not free from the adverse affects of the RIFA. The damage suffered by plants generally results either from the colonizing or foraging behavior of the RIFA, or from the ants feeding directly on the plants. Though it is likely that numerous other plants are affected by these ants, commodity crops have received the most

attention, due to the potential economic repercussions. Commodity crops such as corn, sorghum, eggplants, peanuts, and soybeans are all susceptible to damage or destruction resulting from RIFA activity (Adams 1983; Drees et al. 1991; Shatters and Vander Meer 2003; Vogt et al. 2001).

From a broader perspective than that offered at the organismal level, such as in the previously mentioned examples, it is also oftentimes possible to see the collective effects of the RIFA at the level of the ecosystem. Especially in heavily infested areas, the RIFA has the potential to alter the food web, sometimes drastically. The RIFA achieves this by way of elimination, displacement, or replacement of vital components of one or more trophic levels of the food web, disruption of mutualistic relationships, or even through sometimes-severe over utilization of resources. Indeed, the overwhelming success and proliferation of the RIFA in this country has allowed this invasive species to become influential enough to alter entire ecosystems (Carroll and Hoffman 2000; Folgarait 1998; Gotelli and Arnett 2000; Ness 2003; Porter et al. 1997; Vinson 1994; Zettler et al. 2001).

A major reason for the unprecedented success of the RIFA within the United States, especially in terms of their ever-expanding range and their extraordinary, virtually unchecked competitive advantage over so many native species, is the unique combination of characteristics that these ants possess. However, prior to examining that unique combination of characteristics, it is first necessary to explore the basic biology and ecology behind them. To begin, an integral part of the biology and ecology of the eusocial RIFA is their caste system. The caste system consists of the brood caste (the eggs, larvae, and pupae collectively), the worker caste, and the reproductive caste. In

terms of the brood caste, all members of this caste are white to slightly yellow in color (though the pupae grow progressively darker as they near adulthood), eggs are less than 0.5 mm in length, the four larval instars are highly variable in length, and the pupae are generally longer than 2 mm. The brood caste is subdivided into two different subcategories, worker brood and reproductive brood, and there are numerous differences between the two. The various stages of the worker brood are smaller than the corresponding stages of the reproductive brood, and worker brood will mature into members of the worker caste, while reproductive brood will mature into members of the reproductive caste. Additionally, reproductive brood is mainly produced during the spring and summer, while worker brood is produced year round (Lofgren et al. 1975; Vinson 1997).

The worker caste consists of major workers and minor workers, and all members of this caste are capable of stinging. Members of the worker caste are polymorphic (most commonly 3-5 mm in length), sterile, female, and reddish-brown with a dark colored gaster. Workers also have 10-segmented antennae with a two-segmented club, a two-segmented pedicel, mandibles with four teeth, and a stinger. The functions of the worker caste include sanitation, colony defense, mound repairing and building, finding food for the colony (foragers), and tending to the brood and queen (nurses). The major workers are the larger members of the caste, up to 5 mm in length with a mean head capsule width of 1.5 mm, and they usually spend only a small fraction of their lives as nurses, focusing more on the other aforementioned functions of the worker caste. The minor

workers are smaller, commonly 3 mm in length with a mean head capsule width of 0.5 mm, and they oftentimes spend a large portion of their lives as nurses.

The reproductive caste consists of winged reproductives, virtually all of which are longer than 5 mm. Winged reproductives are either males, with the entire body black, or females, with a reddish brown body and black gaster. Winged reproductives are commonly found within a colony from the spring to the fall, and these caste members swarm during mating flights with the purpose of founding new colonies. The other member of the reproductive caste is the queen, a gravid, dealated reproductive. She is ultimately responsible for the proliferation of the colony via oogenesis and oviposition (Lofgren et al. 1975; Vinson 1997).

New colonies commonly begin as a result of nuptial flights. Dependent upon weather conditions, nuptial flights of the winged reproductives occur from the spring to the fall throughout most of the range of the RIFA, and new colonies are constantly being formed during that time. Nuptial flights occur up to 300 m in the air, and ultimately result in mating between males and females, with the mating actually occurring in flight. Shortly after copulation, the male dies, but the female persists and begins searching for an appropriate site to begin a new colony, usually underneath a rock or leaf litter, or other such sheltered locations. Upon finding an appropriate site, the gravid queen dealates, excavates a small subterranean cell 7-20 cm below the surface, seals it off and, within 24 hours, oviposits.

Within 6-10 days of oviposition, the RIFA eggs hatch. The immature ants then progress through four larval instars and a pupal stage and, at least in newly-founded

colonies, they will all ultimately emerge from the pupal stage as adult workers since new colonies do not begin producing reproductive brood for at least one year. On average, the time that elapses between oviposition and the emergence of the adult stage is 20-30 days, dependent upon temperature; and the typical RIFA worker lives 1-6 months, also dependent upon temperature. The queen of a new colony can lay as many as 2000 eggs a day and this, along with the considerable life span of the workers, allows a RIFA colony to grow at a relatively rapid pace. After 1-3 years of such growth, colonies are considered to be mature, though they can continue to grow for many more years, as the life span of the queen is 6-7 years. A large, mature colony typically consists of a highly variable number of brood, 200,000-300,000 workers, a highly variable number of winged reproductives, and either one queen (monogyne colony) or multiple queens (polygyne colony) (Lofgren et al. 1975; Markin et al. 1973; Vinson 1997).

The colony of the RIFA is an extensive, subterranean, tunnel-filled labyrinth, with tunnels extending 30-40 cm or more down into the soil. Most often, directly above the subterranean portion of the colony is an above ground mound, which can range in height from less than 5 cm to more than 40 cm. The purpose of the above ground mound is to allow the RIFA workers to maintain strict control over brood thermoregulation, moving the brood up or down within this above ground portion as solar radiation and environmental conditions dictate. Many of the colony's tunnels radiate out from this centrally located mound. Typically located 2-12 cm belowground, these tunnels are oriented in parallel fashion to the surface, and terminate in superficial exit holes that may be located as much as 10 m or more away from the mound. It is through these tunnels

that the RIFA foragers move in relative safety while foraging (Markin et al. 1973; Showler et al. 1990; Vinson 1997).

RIFA colonies can be found in numerous habitats and in virtually any soil type, ranging from sandy to clay. There are only a few limiting factors in terms of where these ants are able to colonize. One of these limiting factors is heavily wooded habitats, which the RIFA will generally not colonize due to the fact that the thick canopy does not allow sunlight to penetrate, thus hindering appropriate thermoregulation of the brood within the mound. In addition to heavily wooded habitats, essentially the only other limiting factors are cold temperatures and extremely arid regions that lack a permanent and accessible source of water. Resultantly, the RIFA can be found in virtually any habitat that is relatively open, has a permanent source of food and water, and that has minimum yearly temperatures above -12.3° C. Within such areas, it is common to find 50-75 mounds per hectare, though as many as 150 mounds per hectare have been observed (Lofgren et al. 1975; Vinson 1997).

As far as defending their colonies, there are very few, if any, organisms that are more successful in that endeavor than the RIFA. When a mound is disturbed, a large number of workers quickly exit the mound and attempt to attach themselves, via their mandibles, to the offending organism. Once attached, a RIFA will repeatedly sting the organism until it falls off of or is removed by the organism, or until the organism dies. The venom of the RIFA injected via stinging is poisonous to many organisms, ranging from other arthropods to a wide variety of vertebrates, as it is comprised of toxins containing water insoluble isomers of n-alkyl and n-alkenyl piperidine alkaloids, both of which possess

necrotizing properties. The massive and toxic defensive response of the RIFA is usually enough to either drive an offending organism away, or to kill it. Essentially this same tactic, complete with venomous attack in mass, is also used by the RIFA to subdue and kill prey for food. This tactic is especially effective against other arthropods, due to the fact that the toxins in the RIFA's venom possess insecticidal qualities in addition to the aforementioned necrotizing properties (Lofgren et al. 1975; Stafford 1996; Vinson 1997).

Like their tenacious defensive behavior, foraging behavior is also a very important component of the biology and ecology of the RIFA. As previously mentioned, foraging is one of the duties carried out by a specific, commonly older set of individuals within the worker caste. These individuals are known as foragers, and they are most active when the soil temperature is 21-35° C. The foragers disperse from the colony in search of food via the numerous horizontal tunnels, referred to as foraging tunnels, which extend more than 10 m from the centrally located mound. Once a sizable food source is located, the foragers secrete a trail pheromone as they return to the foraging tunnels. This pheromone lingers, thus creating a trail from the food source to the foraging tunnel, which allows other foragers to locate the source of food. Both liquid foods, which the foragers store in their crop, and solid foods are collected and taken back to the mound. In terms of the specific types of foods that the foragers seek, RIFA are truly omnivorous and feed on other arthropods and invertebrates, small vertebrates, plants, and dead animal and plant tissue. Research has shown that the RIFA will forage greater distances

for carbohydrate sources than for either lipid or protein sources (Lofgren et al. 1975; Porter and Tschinkel 1987; Vinson 1997; Weeks et al. 2004).

While each of the previously mentioned attributes, qualities, behaviors, and activities plays an important role in terms of the biology and ecology of the RIFA, perhaps the most unique attributes of the these ants revolve around their social feeding behavior. To begin, the distribution of all food throughout the entire colony is ultimately accomplished via the process of trophallaxis, although the sequence of distribution is different when comparing liquid foods to solid foods. The general pathway involving liquid foods, such as oils, begins with the foragers first feeding the nurses via trophallaxis, who then feed the larvae and queen. This pathway is relatively simple, as all caste members are capable of digesting liquid foods.

The general pathway involving solid foods is more complex, however, as the only caste members that can digest solid foods are the fourth instar larvae. Using protein as an example of a solid food, the process begins with foragers passing the protein to the nurses, who then pass it along to the fourth instar larvae. These larvae digest the solid protein and, via trophallaxis, pass amino acids back to the nurses. By way of trophallaxis, the nurses then feed the amino acids to the other larval instars, the queen, and the foragers. It is through these two unique trophallaxis-driven pathways, one involving liquid foods and the other involving solid foods, that the entire colony is ultimately supplied with all necessary nutrients (Cassill and Tschinkel 1995; Lofgren et al. 1975; Vinson 1983, 1997).

One characteristic that has allowed the RIFA to be so successful in terms of range expansion is the fact that they have nuptial flights. Though weak fliers, these nuptial flights still commonly allow them to bypass geographical barriers that would likely otherwise be range-limiting. Another trait that has been critical to the success of the range expansion of the RIFA is the fact these ants are so flexible in terms of their habitat. As previously mentioned, virtually any habitat that is relatively open, has a permanent source of food and water, and that has minimum yearly temperatures above -12.3° C is likely to be habitable by the RIFA.

In terms of their oftentimes overwhelming competitive advantage over native species, there are numerous characteristics that have resulted in this advantage. These characteristics include the fact that RIFA are omnivorous, they are general foragers, they attack and defend in aggregate with multiple and debilitating stings, they are capable of reproducing at a prolific rate and, finally, they have the ecological advantage of having very few natural enemies in this country. Collectively, this is the biologically and ecologically-based combination of characteristics that has allowed the RIFA to expand so freely, and to be so fiercely and successfully competitive against the native fauna in this country (Porter and Savignano 1990; Simberloff 1997; Tschinkel 1993; Vinson 1994, 1997).

Unfortunately, the success of the RIFA has been manifested in the form of economic hardship for the United States. It is estimated that the costs of damage associated with the RIFA in sectors ranging from agricultural to urban exceed $680 million annually in the state of Texas alone, and this estimate does not even include damage inflicted upon

the game and wildlife sector (Lard et al. 2001). Considering the fact that the RIFA is also established in 13 other states, it is likely that the overall cost of damage associated with these ants in the United States is billions of dollars annually, and it would likely be many times that without the implementation of control measures. With such staggering costs attributed to damage caused by the RIFA, the need for effective control measures is apparent.

Chemicals have been utilized in an attempt to control the RIFA since shortly after their arrival in this country. Popular throughout the 1930's and 1940's, calcium cyanide was the first chemical used in an attempt to control the RIFA, and this was followed by heptachlor and dieldrin in the 1950's (Eden and Arant 1949; Sauer et al. 1982). These were followed in the 1960's and 1970's by a shift to the use of baits, primarily those containing mirex (Lofgren et al. 1975). Baits were very effective because they took advantage of the previously described complex, trophallaxis-driven, social feeding behavior of the RIFA. These popular baits were commonly composed of corncob grit-based granules, which were coated with some type of oil (most commonly soybean oil) to which the insecticide had been added (Banks 1990; Lofgren et al. 1964). Essentially, this same recipe for baits is still used today, and only the active ingredient has changed. Presently, some of the more commonly-used baits contain such chemicals as hydramethylnon and fenoxycarb (Collins et al. 1992; Phillips and Thorvilson 1989; Vander Meer et al. 1982). It is estimated that in Texas alone, nearly $520 million is spent annually in an effort to control the RIFA, with the majority of that money being spent on chemical control (Lard et al. 2001).

In spite of the fact that the majority of the money that is spent on RIFA control measures is used for chemical control, there are other potential methods of control, such as the use of biological control agents (Nichols and Sites 1991; Whitcomb et al. 1973; Wojcik 1998). Some of the organisms that are currently being studied for use as potential agents for controlling the RIFA include a parasitic ant (*Solenopsis daguerri*), parasitoid phorid flies (*Pseudacteon* spp.), parasitoid wasps (*Orasema* spp.) a microsporidial protozoan (*Thelohania solenopsae*), and a fungus (*Beauveria bassiana*) (Knutson and Drees 1998; Morrison et al. 1997; Williams et al. 1999). The use of biological control agents such as these is appealing, as they are potentially less harmful to the environment than chemicals, and they are oftentimes less likely to pose a danger to non-target organisms. Unfortunately, none of the biological control agents currently available are stand-alone methods of control and, therefore, they must be paired with other methods in order to achieve effective RIFA control (Drees et al. 1996).

Conversely, there are several chemicals currently available that, when used appropriately, are very effective in terms of RIFA control. However, due to a combination of RIFA resiliency and the United States Environmental Protection Agency's increasingly strict regulations, the continuous need to develop new and effective chemical control measures exists. Indoxacarb, chemical name (S)-methyl 7-chloro-2,5-dihydro-2-[[(methoxy-carbonyl)[4(trifluoromethoxy)phenyl]amino]-carbonyl]indeno[1,2-e][1,3,4]oxadiazine-4a-(3H)-carboxylate, is one such novel chemical that is currently being investigated for use in controlling the RIFA. Discovered by E.I. DuPont de Nemours and Company in 1991, indoxacarb is classified as an

oxadiazine, which is a new class of pyrazoline-type insecticides (McCann et al. 2001). Indoxacarb is a novel insecticide that must first be metabolized in order to become acutely toxic. Following ingestion by the insect, metabolic breakdown of indoxacarb occurs via amidase and esterase enzymes commonly found within the midgut and/or fat bodies, ultimately producing an N-decarbomethoxyllated metabolite known as JT333. It is JT333 that is so highly toxic to the insect, functioning as a potent, voltage-dependent, sodium channel blocker. The bio-activation of this potent metabolite within the insect ultimately results in severe neurotoxic symptoms, paralysis, and a relatively rapid death (Wing et al. 2000).

Indoxacarb is attractive as a form of chemical control for numerous reasons, including the fact that it has a novel mode of action, it is broad spectrum, it has low mammalian toxicity, it is effective in many instances against insects resistant to pyrethroids, carbamates, and organophosphates, bio-activation is rapid in sensitive insects, JT333 is very slow to dissociate from the sodium channel once it is bound, and death occurs rapidly in sensitive insects (Wing et al. 2000). Though apparently not all insects are sensitive to indoxacarb, it has proven to be extremely effective at controlling a wide variety of the insects upon which it has been tested thus far, including pest lepidopterans and hemipterans, and it is especially effective when ingested, as opposed to topically applied (Hewa-Kapuge et al. 2003; Liu et al. 2002, 2003; Nowak et al. 2001; Tillman et al. 2001; Wing et al. 1998, 2000). Because of the numerous positive attributes associated with indoxacarb, such as those aforementioned, and because of the

success of this chemical against lepidopterans and hemipterans, research on the effects of this chemical on different types of insects continues.

Some of the most recent indoxacarb-related research has been conducted on the RIFA. Preliminary results of the effects of indoxacarb on the RIFA were positive enough that in August of 2004, E.I. DuPont de Nemours and Company in Wilmington, Delaware, sought and received approval from the United States Environmental Protection Agency for the label of a new RIFA control product: Advion™ (EPA Registration #352-627). Advion™ is a corncob grit-based, granular bait that contains indoxacarb as the active ingredient. Because it has only recently been introduced into the marketplace, limited research on Advion™ has been conducted and published to date. Therefore, the purpose of this research was to evaluate the new RIFA bait Advion™.

Specifically, this research consisted of five objectives: 1) to evaluate numerous technical aspects of Advion™ in an attempt to ensure that the bait is maximally effective against the RIFA in terms of overall mortality and speed of mortality (Chapter II); 2) to determine the most appropriate specifications for individual mound treatments with Advion™ in an attempt to ensure maximum control of individual RIFA colonies with such treatments (Chapter III); 3) to evaluate and compare the effectiveness of label-rate broadcast treatments with Advion™ to the effectiveness of other broadcast treatments aimed at RIFA control (Chapter IV); 4) to evaluate the trophallactic transmission and metabolism of indoxacarb by the RIFA (Chapter V); and 5) to evaluate the effectiveness

of label-rate broadcast treatment with Advion™ at controlling multiple ant species (Chapter VI).

In terms of the first objective, evaluation of numerous technical aspects of Advion™, this research was focused on the determination of three factors: the most effective chemical form of indoxacarb, the most effective concentrations of indoxacarb, and the most appropriate grit size for use in Advion™. With relation to chemical form, no research has previously been conducted which compares the efficacy of Advion™ containing indoxacarb to the efficacy of Advion™ containing JT333. Additionally, no research has been conducted in an attempt to determine the efficacy of varying concentrations of indoxacarb in Advion™. As far as grit size, limited research has previously been conducted which analyzed grit size preferences for numerous urban ant species (Hooper-Bui et al. 2002). However, no previous research has attempted to determine RIFA size preferences of corncob grit containing active ingredient, specifically grit containing indoxacarb. Therefore, this research was conducted in an attempt to determine the most effective chemical form and concentrations of indoxacarb, as well as the most appropriate grit size, for use in Advion™ to ensure that the bait is maximally effective against the RIFA in terms of overall mortality and speed of mortality.

For the second objective, research was conducted to determine the most appropriate specifications for individual RIFA mound treatments with Advion™, which is an area in which research has not previously been conducted and published. To achieve this objective, experimentation was conducted in an attempt to determine the most effective

concentrations and quantities of Advion™ for use with individual RIFA mound treatments, and to determine the effects of placing the bait at varying distances from the mound during individual mound treatments. Ultimately, this experimentation was conducted to ensure that the specifications for individual RIFA mound treatments with Advion™ are such that maximum control of individual RIFA colonies is achieved.

With relation to the third objective, comparing the effectiveness of broadcast treatment with Advion™ to other broadcast treatments, only limited research comparing the effectiveness of label-rate broadcast treatment with Advion™ to the effectiveness of label-rate broadcast treatment with other RIFA baits has previously been conducted and published (Barr 2003). Furthermore, no research has been conducted and published on the effectiveness of Advion™, or any other RIFA bait, when used in a pre-baiting broadcast treatment. Pre-baiting, which involves treatment with non-toxic bait in order to stimulate feeding activity prior to treatment with toxic bait, has already proven to be effective at controlling numerous pest species (Shumake et al. 2002, Sterner 1999). Resultantly, this research was conducted to compare the effectiveness of label-rate broadcast treatment with Advion™ to the effectiveness of both pre-baiting broadcast treatment with Advion™, and label-rate broadcast treatment with the popular RIFA bait Amdro® (Ambrands, Atlanta, GA).

In terms of the fourth objective, evaluation of the trophallactic transmission and metabolism of indoxacarb by the RIFA, no research has been conducted that specifically illustrates the trophallactic transmission of indoxacarb. Furthermore, while the metabolism of indoxacarb has been well studied in other insects, and it is believed that,

within a RIFA colony, the larvae are responsible for the metabolism of indoxacarb into the toxic JT333, no research has been conducted and published with relation to the ability of RIFA workers to metabolize indoxacarb (E.I. DuPont de Nemours and Company 2004; Wing et al. 2000). Therefore, utilizing Advion™ as the source of indoxacarb, this research was conducted to illustrate trophallactic transmission of indoxacarb and to analyze the ability of members of the RIFA worker caste to metabolize indoxacarb.

With relation to the fifth and final objective, evaluation of the effectiveness of a label-rate broadcast treatment with Advion™ at controlling multiple ant species, no research has previously been conducted or published. Though limited research has been conducted to evaluate the effectiveness of label-rate broadcast treatment with Advion™ at controlling the RIFA, no research has been conducted to evaluate the effects of such treatment on ant species other than the RIFA. Resultantly, this research attempted to address that gap in knowledge by evaluating the effectiveness of label-rate broadcast treatment with Advion™ at controlling multiple ant species in addition to the RIFA, specifically *Monomorium pharaonis*, *Pogonomyrmex barbatus*, and *Dorymyrmex pyramicus*.

CHAPTER II

DETERMINATION OF THE MOST EFFECTIVE CHEMICAL FORM AND CONCENTRATIONS OF INDOXACARB, AS WELL AS THE MOST APPROPRIATE GRIT SIZE, FOR USE IN ADVION™

Introduction

Chemicals ranging from calcium cyanide to fenoxycarb have been used in an attempt to control the red imported fire ant (RIFA), *Solenopsis invicta* Buren, since its accidental introduction into the United States in the 1930's (Banks 1990; Collins et al. 1992; Eden and Arant 1949; Lofgren et al. 1964, 1975; Phillips and Thorvilson 1989; Sauer et al. 1982; Vander Meer et al. 1982). The newest chemical available for the control of the RIFA is indoxacarb. Discovered by E.I. DuPont de Nemours and Company in 1991, the chemical indoxacarb is classified as an oxadiazine, which is a new class of pyrazoline-type insecticides (McCann et al. 2001). Indoxacarb is a novel insecticide that must first be metabolized in order to become acutely toxic. Following ingestion by the insect, metabolic breakdown of indoxacarb occurs via amidase and esterase enzymes commonly found within the midgut and/or fat bodies, ultimately producing an N-decarbomethoxyllated metabolite known as JT333. It is JT333 that is so highly toxic to the insect, functioning as a potent, voltage-dependent, sodium channel blocker. The bio-activation of this potent metabolite within the insect ultimately results

in severe neurotoxic symptoms, paralysis, and a relatively rapid death (Wing et al. 2000).

One of the newest products available for the control of the RIFA is Advion™ (E.I. DuPont de Nemours and Company, Wilmington, DE), which contains the active ingredient indoxacarb. Registered by the United States Environmental Protection Agency in August of 2004 (EPA Registration #352-627), Advion™ is a corncob grit-based, granular bait. As with any RIFA bait, all aspects of Advion™ should be evaluated to ensure that it is maximally effective at controlling the RIFA. However, being new to the marketplace, little research on Advion™ has been conducted and published (Barr 2003). Therefore, this research was conducted to evaluate several technical aspects of the new RIFA bait Advion™, in an attempt to ensure that the bait is maximally effective against the RIFA in terms of overall mortality and speed of mortality.

Specifically, this research was focused on the determination of three factors: the most effective chemical form of indoxacarb, the most effective concentrations of indoxacarb, and the most appropriate grit size for use in Advion™. First, with relation to chemical form, no research has previously been conducted which compares the efficacy of Advion™ containing indoxacarb to the efficacy of Advion™ containing JT333. As previously mentioned, indoxacarb is not believed to be acutely toxic until it is bio-activated via metabolic breakdown into JT333 (Wing et al. 2000). Since it is actually JT333 that is acutely toxic, it was necessary to determine which would be more effective

at controlling the RIFA: Advion™ containing indoxacarb, or Advion™ containing JT333.

No research has previously been conducted with the purpose of determining the efficacy of varying concentrations of indoxacarb in Advion™ and, therefore, this research need exists. Also, while limited research has previously been conducted which analyzed corncob grit size preferences for numerous urban ant species (Hooper-Bui et al. 2002), no previous research has attempted to determine RIFA size preferences of corncob grit containing active ingredient, specifically grit containing indoxacarb. Therefore, experimentation was necessary to determine the Advion™ grit size that would most likely be carried back to the colony by RIFA foragers. Summarily, the objective of this research was to determine the most effective chemical form and concentrations of indoxacarb, as well as the most appropriate grit size, for use in Advion™ in an attempt to ensure that the bait is maximally effective against the RIFA in terms of overall mortality and speed of mortality.

Materials and Methods

The following three experiments were all conducted in the laboratory, and procedures for RIFA collection and pre-experiment maintenance of the ants were identical for each. All of the RIFA colonies used in these experiments were collected from the USDA-ARS Pecan Breeding Orchard (N30°37’21” W96°21’34”) located in Brazos County, Texas, which was chosen as the collection site due to the fact that no pesticides have been used on the land. Prior to each experiment, several RIFA colonies were excavated and placed

into 12 liter plastic buckets that had been lined with talcum powder to prevent the ants from escaping. The colonies were transported back to the laboratory, dripped out, and then placed into 40 cm x 27 cm x 9.5 cm plastic sweater boxes (First Phillips Manufacturing, Leominster, MA) that were lined with Fluon® (Northern Products, Inc., Woonsocket, RI) to prevent the ants from escaping (Sorensen and Vinson 1981; Weeks et al. 2004).

Each sweater box contained a 14 cm x 2.5 cm petri dish, a 7.5 cm x 2 cm plastic weigh dish, and two 4 cm x 0.8 cm plastic weigh dishes. The petri dish served as the artificial brood chamber for the ants. It was filled with 1.5 cm of Castone® Dental Stone (Dentsply International, York, PA), which had been moistened with water prior to placing the ants into the sweater box, and the plastic lid on top of the petri dish contained two 3 cm holes which been cut into the lid to allow the RIFA easy access to the brood chamber. The large weigh dish inside the sweater box contained cotton saturated with water, while one small weigh dish contained cotton saturated with a 20% sugar water solution, and the other small weigh dish contained live tenebrionid beetle larvae (Banks et al. 1981; Cassill and Tschinkel 1999; Sorensen and Vinson 1981).

Immediately after the ants had been placed into their respective sweater boxes, each colony was tested to ensure that none of the colonies that were to be used in the experiment contained the microsporidial protozoan, *Thelohania solenopsae*. The presence of these protozoans could indicate an unhealthy colony, and the use of such a colony in a laboratory experiment would likely provide unreliable data. In order to test for the presence of *T. solenopsae,* 30 workers were taken from a given colony, placed in

a 0.5 ml Eppendorf® microcentrifuge tube (Eppendorf North America, Westbury, NY) to which 50 µl of deionized water had been added via a Rainin® EDP2™ 25-250 µl electronic pipette (Rainin Instrument, LLC, Oakland, CA), and macerated with an Eppendorf® micropestle (Eppendorf North America, Westbury, NY). Then, 30 µl of the newly formed homogenate was placed on a 2.5 cm x 7.5 cm x 1.0 mm glass slide and allowed to air-dry for 24 h (Keck 2005). After 24 h, the staining steps as outlined by Weber et al. (1992) were followed; and after another air-drying period of ~24 h, it was then possible to view the slide under a light microscope and determine whether or not *T. solenopsae* spores were present. The entire testing procedure took ~2 d, and any colonies in which *T. solenopsae* were found were not used.

After testing all colonies for the presence of *T. solenopsae*, sufficient numbers of colonies were used for treatment and control groups required for the experiment. Separate colonies were used for each treatment group and control group. Three replicates were used per group, and both RIFA workers and brood were used in each replicate. Three groups of workers and brood from each of the selected colonies were weighed and placed into separate sweater boxes. From each colony, three groups consisting of 5 g of workers (~10,000 workers) and 1.25 g of brood (~2500 brood) each were weighed on an Ainsworth® 6000 g electric scale, model APX-6001 (Denver Instrument Company, Denver, CO), and placed into each of three Fluon®-lined sweater boxes. Ultimately, this yielded a total of three replicates per treatment and control group, with each replicate consisting of ~10,000 workers and ~2500 brood. The new sweater boxes into which RIFA were placed contained the same materials as the sweater

boxes that originally contained each entire colony: a petri dish, a large weigh dish, and two small weigh dishes. The only difference was that the petri dishes in the new sweater boxes were smaller, at 9 cm x 1.5 cm, and they contained 0.75 cm of Castone® dental stone.

All of the RIFA that were used in these experiments were maintained in a manner similar to that described by Banks et al. (1981). This included keeping them in an environment of continuous light at a constant temperature of ~28° C, providing them with a constant supply of water, and maintaining them on a diet of 20% sugar water solution and tenebrionid beetle larvae. The RIFA were maintained in this manner for a total of 5 d to allow the ants a sufficient amount of time to become acclimated to the laboratory conditions (Collins and Callcott 1998). At the end of day 5, the small weigh dishes that contained food were removed from each sweater box. The RIFA were starved for 2 d to ensure that the ants' crops were emptied (Cassill and Tschinkel 1999).

Chemical Form. Experimentation comparing the efficacy of Advion™ containing indoxacarb to the efficacy of Advion™ containing the toxic JT333 was conducted for 30 d (June 21-July 21, 2004). A single RIFA colony was used for each of the three different treatment groups, as well as the control group, of which this experiment was comprised. Thus, a total of four RIFA colonies were utilized. As aforementioned, there were three replicates per group. All of the bait utilized for this experiment was freshly produced and shipped directly from E.I. DuPont de Nemours and Company's research laboratory in Newark, Delaware.

On the first day of the experiment, one small weigh dish was placed into each of the three sweater boxes for Treatment 1, Treatment 2, and the control group, and two small weigh dishes were placed into each of the three sweater boxes for Treatment 3. Each small weigh dish contained 1 g of Advion™ with 0.045% indoxacarb for Treatment 1, 1 g of Advion™ with 0.045% JT333 for Treatment 2, and 1 g of 0.0% Advion™ (contained no indoxacarb and no JT333) for the control group. For Treatment 3, two small weigh dishes were placed into each sweater box: one containing 1 g of Advion™ with 0.045% indoxacarb, and the other containing 1 g of Advion™ with 0.045% JT333. Each of the sweater boxes for Treatment 1, Treatment 2, and the control group contained an artificial brood chamber, a large weigh dish containing water-soaked cotton, and a small weigh dish containing 1 g of the appropriate bait (Figure 1). Each of the sweater boxes for Treatment 3 contained an artificial brood chamber, a large weigh dish containing water-soaked cotton, and two small weigh dishes, each containing 1 g of the appropriate bait (Figure 2).

Over the course of the experiment, three factors were measured: feeding activity, mortality, and the amount of bait removed from the weigh dish. In terms of feeding activity, this was measured hourly for the first 5 h of the experiment, and then once daily for the duration of the experiment. Feeding activity was measured by counting the number of RIFA that were actually in the weigh dish feeding on the bait at the time of observation. With relation to mortality, the number of dead RIFA workers was estimated once daily. Finally, to determine the amount of bait that had been removed from the weigh dishes by the RIFA workers and taken into the brood chamber, the small

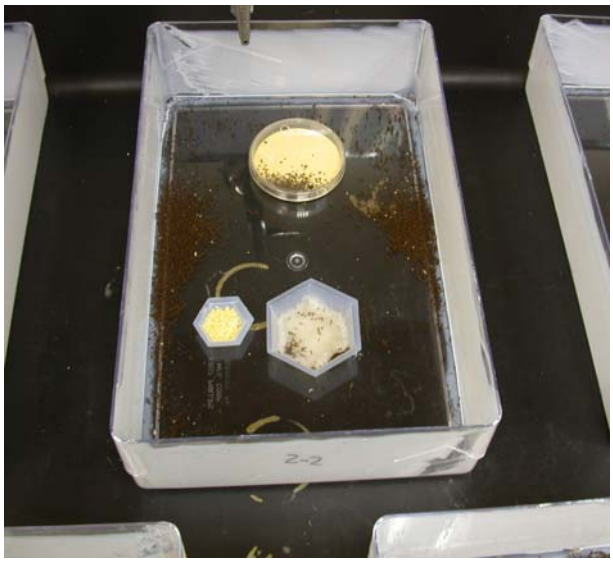

Figure 1. A Fluon®-lined sweater box containing an artificial brood chamber, ~10,000 RIFA workers, ~2500 brood, a large weigh dish with water-soaked cotton, and a small weigh dish with 1 g of Advion™ containing 0.045% indoxacarb.

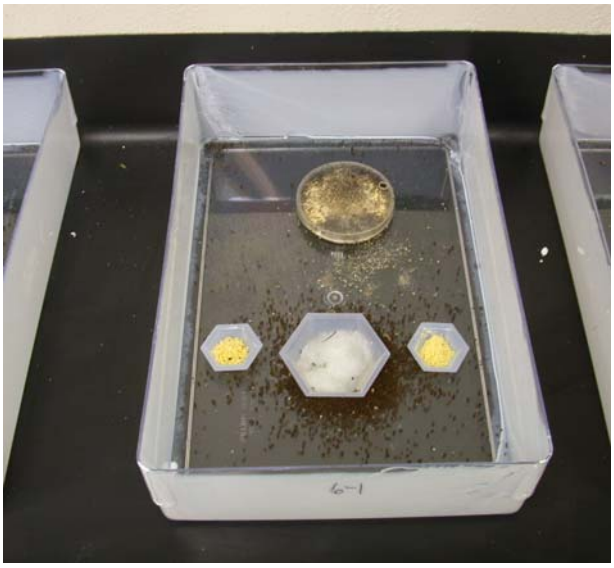

Figure 2. A Fluon®-lined sweater box containing an artificial brood chamber, ~10,000 RIFA workers, ~2500 brood, a large weigh dish with water-soaked cotton, a small weigh dish with 1 g of Advion™ containing 0.045% indoxacarb (left), and a small weigh dish with 1 g of Advion™ containing 0.045% JT333 (right).

weigh dishes were removed from each of the sweater boxes every 5 d, weighed on an Ainsworth® 6000 g electric scale, model APX-6001 (Denver Instrument Company, Denver, CO), and then placed back into the appropriate box. All measurements for this experiment were conducted at approximately the same time each day.

Concentrations. Experimentation comparing the efficacy of different concentrations of indoxacarb in Advion™ was conducted for 30 d (June 21-July 21, 2004). A single RIFA colony was used for each of the five different treatment groups, as well as the control group, of which this experiment was comprised. Thus, a total of six RIFA colonies were utilized. As with the previous experiment, there were three replicates per group. All of the bait utilized for this experiment was freshly produced and shipped directly from E.I. DuPont de Nemours and Company's research laboratory in Newark, Delaware.

On the first day of the experiment, one small weigh dish was placed into each of the three sweater boxes for the five different treatment groups and the control group. Each small weigh dish contained 1 g of 0.10% Advion™ for Treatment 1, 1 g of 0.06% Advion™ for Treatment 2, 1 g of 0.045% Advion™ for Treatment 3, 1 g of 0.015% Advion™ for Treatment 4, 1 g of 0.001% Advion for Treatment 5, and 1 g of 0.0% Advion™ (contained no indoxacarb) for the control group. Resultantly, each of the 18 sweater boxes contained an artificial brood chamber, a large weigh dish containing water-soaked cotton, and a small weigh dish containing 1 g of the appropriate bait. Over the course of the experiment, three factors were measured: feeding activity, mortality,

and the amount of bait removed from the weigh dish. Each of these three factors was measured in a manner identical to that described in the previous experiment.

Grit Size. Experimentation comparing RIFA preference for different Advion™ grit sizes was conducted for 30 d (August 26-September 25, 2004). A single RIFA colony was used for each of the three different treatment groups and the three different control groups of which this experiment was comprised. Thus, a total of six RIFA colonies were utilized. As with the previous experiments, three replicates were used per group. For this experiment, two different grit sizes were utilized: standard sized grit and small sized grit. The standard sized grit (~2 mm/granule), which is the size currently found in Advion™ and several other corncob grit-based RIFA baits, consisted of ~600 granules per gram, while the small sized grit (<1 mm/granule) consisted of ~3000 granules per gram (Figure 3). A concentration of 0.025% active ingredient was used for all treatment groups in this experiment due to the fact that this was the highest concentration of indoxacarb that could effectively be placed on the small sized grit. All of the bait utilized for this experiment was freshly produced and shipped directly from E.I. DuPont de Nemours and Company's research laboratory in Newark, Delaware.

On the first day of the experiment, one small weigh dish was placed into each of the three sweater boxes for Treatment 1, Treatment 2, Control 1, and Control 2, while two small weigh dishes were placed into each of the three sweater boxes for Treatment 3 and Control 3. Each small weigh dish contained 1 g of 0.025% Advion™ with standard sized grit for Treatment 1, 1 g of 0.025% Advion™ with small sized grit for Treatment 2, 1 g of 0.0% Advion™ (contained no indoxacarb) with standard sized grit for Control

Figure 3. A comparison of 0.025% Advion™ with small sized grit (<1 mm/granule, pictured on the left) and 0.025% Advion™ with standard sized grit (~2 mm/granule).

1, and 1 g of 0.0% Advion™ (contained no indoxacarb) with small sized grit for Control 2. For Treatment 3, two small weigh dishes were placed into each sweater box: one containing 1 g of 0.025% Advion™ with standard sized grit, and the other containing 1 g of 0.025% Advion™ with small sized grit. Similarly, for Control 3, two small weigh dishes were placed into each sweater box: one containing 1 g of 0.0% Advion™ with standard sized grit, and the other containing 1 g of 0.0% Advion™ with small sized grit. Each sweater box for Treatment 1, Treatment 2, Control 1, and Control 2 contained an artificial brood chamber, a large weigh dish containing water-soaked cotton, and a small weigh dish containing 1 g of the appropriate bait. Each sweater box for Treatment 3 and Control 3 contained an artificial brood chamber, a large weigh dish containing water-

soaked cotton, and two small weigh dishes, each containing 1 g of the appropriate bait. Over the course of the experiment, three factors were measured: feeding activity, mortality, and the amount of bait removed from the weigh dish. Each of these three factors was measured in a manner identical to that described in the previous experiment.

Statistics. At the conclusion of each experiment, SPSS® software (SPSS 2001) was used to conduct statistical analysis of the data. First, however, Abbott's formula was used to correct all mortality data (Abbott 1925). Abbott's formula for correcting mortality data is

$$[(X-Y) / X] \times 100 = \text{percent control}$$

where "X" is the percent survival in the control group, and "Y" is the percent survival in the treatment group. Next, ANOVA was conducted on the feeding activity data set, the corrected mortality data set, and the amount of bait removed data set. Finally, the LSD post hoc test was conducted on each of the three data sets to determine significant differences among treatments. All tests of significance were evaluated at $P = 0.05$.

Results

Chemical Form. A comparison among treatments of the mean cumulative daily mortality is shown in Table 1 and Figure 4 ($F=1.04$; $df=89$; $P<0.05$). Treatment with only Advion™ containing JT333 resulted in significantly greater ($P<0.05$) RIFA mortality for the first 3 d than did treatment with only Advion™ containing indoxacarb,

Table 1. Comparison of the mean percent RIFA mortality resulting from treatment with Advion™ containing 0.045% indoxacarb and Advion™ containing 0.045% JT333.

Day	Mean cumulative percent mortality per treatment[a] (mean ± SD)		
	Treatment 1- Advion™ containing 0.045% indoxacarb	Treatment 2- Advion™ containing 0.045% JT333	Treatment 3- Advion™ containing 0.045% indoxacarb and Advion™ containing 0.045% JT333
1	1.3±1.0a	48.0±2.9b	16.1±2.9c
2	47.1±2.9a	63.8±5.0b	35.3±2.9c
3	63.8±5.0a	70.5±2.9b	53.6±5.0c
4	76.6±2.9a	71.6±2.6a	58.0±5.0b
5	82.2±2.1a	71.6±2.6b	62.0±4.1c
6	86.2±1.6a	71.6±2.6b	65.0±2.5c
7	88.9±1.6a	72.0±2.3b	67.3±3.0b
8	90.9±1.6a	72.0±2.3b	69.0±3.1b
9	91.5±1.0a	72.0±2.3b	69.2±3.0b
10	92.6±0.7a	72.3±2.6b	69.5±2.7b
11	93.0±1.0a	72.3±2.6b	69.5±2.7b
12	93.0±1.0a	72.3±2.6b	69.8±2.7b
13	93.0±1.0a	72.5±2.2b	70.0±3.0b
14	93.2±0.7a	72.5±2.2b	70.0±3.0b
15	93.6±0.9a	72.8±2.4b	70.0±3.0b

[a] Mortality was corrected by the formula in Abbott (1925). Means within a row with different letters are significantly different at $P<0.05$. Means were separated using the LSD test.

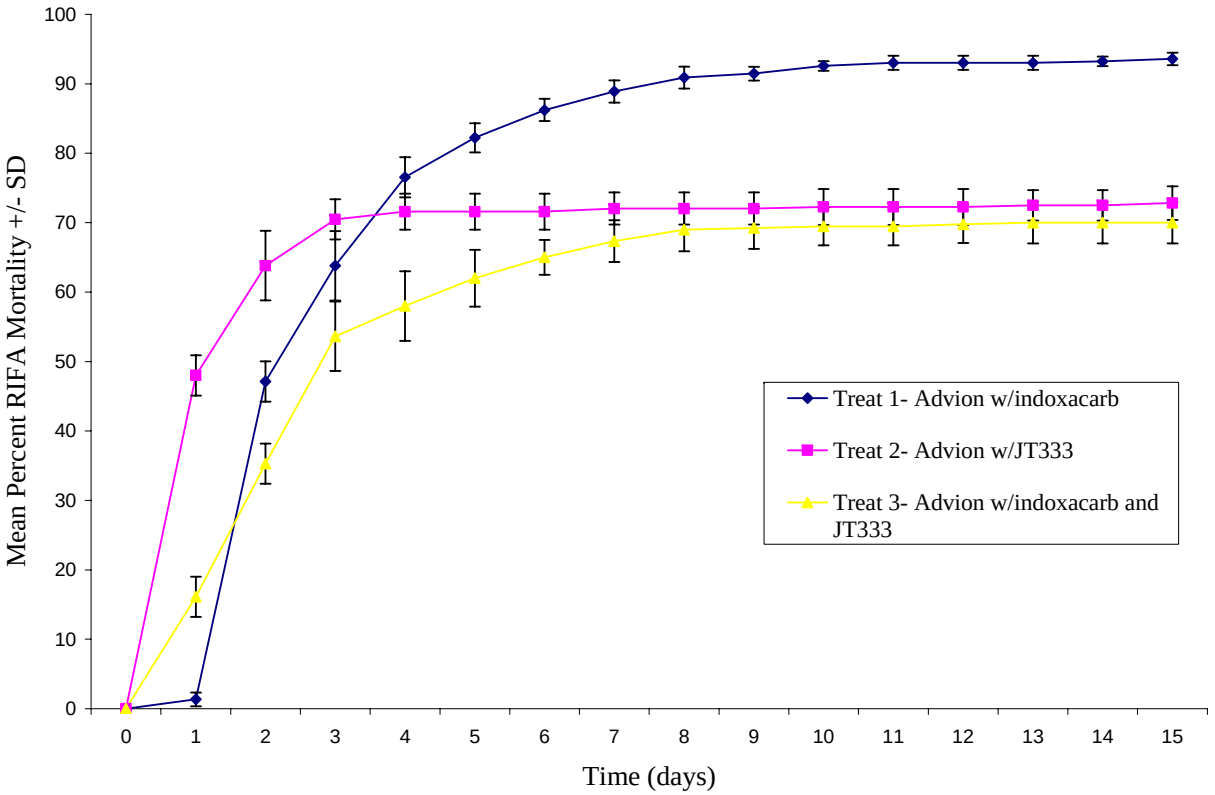

Figure 4. A comparison of the mean percent RIFA mortality resulting from treatment with Advion™ containing 0.045% indoxacarb and Advion™ containing 0.045% JT333.

or treatment with both Advion™ containing indoxacarb and Advion™ containing JT333. However, by the fourth day, there was no significant difference ($P>0.05$) in RIFA mortality between treatment with only Advion™ containing indoxacarb and treatment with only Advion™ containing JT333, and from day 5 through the end of the experiment treatment with only Advion™ containing indoxacarb resulted in significantly greater ($P<0.05$) mortality than did the other two treatments. While treatment with both Advion™ containing indoxacarb and Advion™ containing JT333 resulted in significantly less ($P<0.05$) RIFA mortality than the other two treatments for the first 6 d of the experiment, from day 7 through the end of the experiment there was no significant difference ($P>0.05$) in mortality between treatment with only Advion™ containing JT333 and treatment with both Advion™ containing indoxacarb and Advion™ containing JT333.

A comparison of the LT_{50}'s and LT_{90}'s among treatments is shown in Table 2. The LT_{50} for treatment with only JT333 is, at 1 d, less than 50% of the LT_{50} for treatment with only Advion™ containing indoxacarb (LT_{50}=2.5 d), and less than 33% of the LT_{50} for treatment with both Advion™ containing indoxacarb and Advion™ containing JT333 (LT_{50}=3.4 d). Further, at 7.8 d, treatment with only Advion™ containing indoxacarb had the lowest LT_{90}, as the other two treatments did not attain an LT_{90} over the course of this experiment.

A comparison among treatments of RIFA feeding activity is shown in Table 3 ($F=6.38$; $df=174$; $P<0.05$). When comparing treatment with only Advion™ containing indoxacarb to treatment with only Advion™ containing JT333, there was no significant

Table 2. Comparison of the LT_{50}'s and the LT_{90}'s for RIFA that were treated with Advion™ containing 0.045% indoxacarb and Advion™ containing 0.045% JT333.

	LT_{50} and LT_{90} per treatment (d)		
Lethal time	Treatment 1- Advion™ containing 0.045% indoxacarb	Treatment 2- Advion™ containing 0.045% JT333	Treatment 3- Advion™ containing 0.045% indoxacarb and Advion™ containing 0.045% JT333
LT_{50}	2.5	1.0	3.4
LT_{90}	7.8	—[a]	—[a]

[a] An LT_{90} was not attained during this experiment.

Table 3. Comparison of feeding activity of the RIFA on Advion™ containing 0.045% indoxacarb, Advion™ containing 0.045% JT333, and 0.0% Advion™ (no indoxacarb or JT333). Treatments 1, 2, and the control were all no-choice tests. Treatment 3 was a choice test.

	Mean number of ants feeding in weigh dish per treatment[a] (mean ± SD)				
Time (h)	Treatment 1-Advion™ containing 0.045% indoxacarb	Treatment 2- Advion™ containing 0.045% JT333	Treatment 3- Advion™ containing 0.045% indoxacarb	Treatment 3- Advion™ containing 0.045% JT333	Control- 0.0% Advion™
1	38.3±5.1a	44.7±5.1a	22.3±2.3b	31.0±3.0c	56.0±3.6d
2	46.7±4.7a	50.3±5.0a	30.0±2.7b	35.7±4.2b	77.0±4.6c
3	45.3±4.2a	47.3±5.1a	30.7±1.2b	31.3±1.2b	71.7±4.0c
4	44.3±4.0a	50.7±5.1a	31.0±1.7b	32.7±4.6b	68.0±1.7c
5	44.0±2.0a	44.0±5.3a	27.7±4.2b	29.0±4.6b	67.7±5.1c
24	30.0±1.0a	3.0±5.2b	2.7±3.8b	4.7±4.5b	38.0±6.6c
48	0.3±0.6a	0.3±0.6a	0.0±0.0a	0.0±0.0a	40.3±4.0b
72	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	38.0±4.4b
96	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	35.0±2.6b
120	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	32.7±1.2b

[a] Means within a row with different letters are significantly different at $P<0.05$. Means were separated using the LSD test.

difference (P>0.05) in feeding activity for six of the seven observation periods during which feeding occurred. Likewise, when comparing feeding activity of RIFA that were treated with both Advion™ containing indoxacarb and Advion™ containing JT333, there was no significant difference (P>0.05) in feeding activity on the two different forms of Advion™ for six of the seven observation periods during which feeding occurred. For all observation periods, feeding activity on the 0.0% Advion™ was significantly greater (P<0.05) than that for any of the three treatment groups, and feeding activity had ceased by 72 h for all RIFA except for those in the control group, which continued feeding upon the 0.0% Advion™ for the duration of the experiment. Feeding activity was greatest for all ants during the first 5 h of the experiment.

A comparison among treatments of the amount of bait removed from the weigh dishes is shown in Table 4 (F=3.31; df=29; P<0.05). There was no significant difference (P>0.05) in the amount of bait removed from the weigh dishes by the RIFA for any of the three treatments in which the ants were treated with Advion™ containing active ingredient. Conversely, a significantly greater (P<0.05) amount of bait was removed from the weigh dishes by the RIFA in the control group. No bait was removed from the weigh dishes by the RIFA in any of the treatment groups or the control group following the first weighing event.

Concentrations. A comparison among treatments of the mean cumulative daily mortality is shown in Table 5 and Figure 5 (F=3.88; df=149; P<0.05). When comparing treatment with 0.10% Advion™ to treatment with 0.06% Advion™, there was no significant difference (P>0.05) in RIFA mortality between these treatments for all but

Table 4. Weight-based comparison of the mean percentage of Advion™ containing 0.045% indoxacarb, Advion™ containing 0.045% JT333, and 0.0% Advion™ (no indoxacarb or JT333) that was removed from the weigh dish by the RIFA. Treatments 1, 2, and the control were all no-choice tests. Treatment 3 was a choice test.

Day	Mean cumulative percentage of bait removed from weigh dish per treatment[a] (mean ± SD)				
	Treatment 1- Advion™ containing 0.045% indoxacarb	Treatment 2- Advion™ containing 0.045% JT333	Treatment 3- Advion™ containing 0.045% indoxacarb	Treatment 3- Advion™ containing 0.045% JT333	Control- 0.0% Advion™
5	13.3±5.8a	13.3±5.8a	16.7±5.8a	20.0±0.0a	76.7±5.8b
10	13.3±5.8a	13.3±5.8a	16.7±5.8a	20.0±0.0a	76.7±5.8b
15	13.3±5.8a	13.3±5.8a	16.7±5.8a	20.0±0.0a	76.7±5.8b
20	13.3±5.8a	13.3±5.8a	16.7±5.8a	20.0±0.0a	76.7±5.8b
25	13.3±5.8a	13.3±5.8a	16.7±5.8a	20.0±0.0a	76.7±5.8b
30	13.3±5.8a	13.3±5.8a	16.7±5.8a	20.0±0.0a	76.7±5.8b

[a] Means within a row with different letters are significantly different at $P<0.05$. Means were separated using the LSD test.

Table 5. Comparison of the mean percent RIFA mortality resulting from treatment with 0.10%, 0.06%, 0.045%, 0.015% and 0.001% Advion™.

Day	Mean cumulative percent mortality per treatment[a] (mean ± SD)				
	Treatment 1- 0.10% Advion™	Treatment 2- 0.06% Advion™	Treatment 3- 0.045% Advion™	Treatment 4- 0.015% Advion™	Treatment 5- 0.001% Advion™
1	0.6±0.7a	1.3±1.0a	1.3±1.0a	0.7±0.6a	0.9±0.8a
2	54.1±4.7a	52.2±2.9a	47.1±2.9b	10.1±3.0c	1.9±1.0d
3	74.1±4.7a	68.9±5.0b	63.8±5.0c	25.1±2.9d	3.6±1.4e
4	84.5±1.6a	80.9±2.6a	76.6±2.9b	32.8±5.0c	7.3±2.2d
5	87.8±1.0a	83.4±1.1b	82.2±2.1b	37.5±5.0c	10.3±2.5d
6	89.5±0.5a	86.7±0.9a	86.2±1.6a	44.2±2.9b	12.9±2.9c
7	90.8±0.7a	87.8±1.7a	88.9±1.6a	50.5±1.5b	14.6±2.7c
8	92.8±0.4a	89.2±0.2a	90.9±1.6a	57.2±1.0b	16.6±2.9c
9	93.1±0.4a	90.2±0.7a	91.5±1.0a	61.9±1.5b	19.0±3.4c
10	93.1±0.4a	90.4±0.7a	92.6±0.7a	65.9±1.2b	20.3±2.9c
11	93.3±0.7a	90.6±0.4a	93.0±1.0a	69.9±2.3b	21.3±3.4c
12	93.6±0.3a	91.0±0.3a	93.0±1.0a	72.2±2.6b	22.0±2.5c
13	93.6±0.3a	91.2±0.5a	93.0±1.0a	73.9±2.6b	24.3±2.9c
14	94.1±0.5a	91.2±0.5a	93.2±0.7a	74.7±3.0b	25.9±3.5c
15	94.1±0.5a	91.2±0.5a	93.6±0.9a	75.7±3.7b	28.2±3.6c
16	94.4±0.4a	91.8±0.4a	93.9±1.2a	75.7±3.7b	29.9±3.3c
17	94.4±0.4a	92.0±0.3a	93.9±1.2a	75.8±3.5b	32.2±4.3c
18	94.6±0.7a	92.2±0.2a	94.1±0.8a	76.2±3.8b	33.9±4.3c
19	95.1±0.4a	92.2±0.2a	94.1±0.8a	76.2±3.8b	35.0±4.5c
20	95.1±0.4a	92.8±0.2a	94.4±1.0a	76.7±3.6b	36.3±5.1c
21	95.1±0.4a	93.1±0.2a	94.4±1.0a	76.9±3.8b	36.8±4.9c
22	95.1±0.4a	93.1±0.2a	94.6±0.6a	76.9±3.8b	38.1±4.6c
23	95.7±1.0a	93.4±0.5a	95.0±0.8a	77.6±2.7b	39.8±5.2c
24	96.1±0.4a	93.4±0.5a	95.4±1.0a	77.9±2.9b	41.8±5.2c
25	96.1±0.4a	93.6±0.3a	95.4±1.0a	78.1±3.2b	42.6±5.9c
26	96.1±0.4a	93.9±0.4a	95.4±1.0a	78.1±3.2b	43.1±5.3c
27	96.1±0.4a	93.9±0.4a	95.4±1.0a	78.5±2.6b	44.5±5.5c
28	96.3±0.0a	94.1±0.3a	95.4±1.0a	78.5±2.6b	45.2±4.1c
29	96.3±0.0a	94.1±0.3a	95.4±1.0a	78.5±2.6b	46.2±4.1c
30	96.5±0.4a	94.3±0.0a	95.4±1.0a	79.0±3.0b	46.5±4.1c

[a] Mortality was corrected by the formula in Abbott (1925). Means within a row with different letters are significantly different at $P<0.05$. Means were separated using the LSD test.

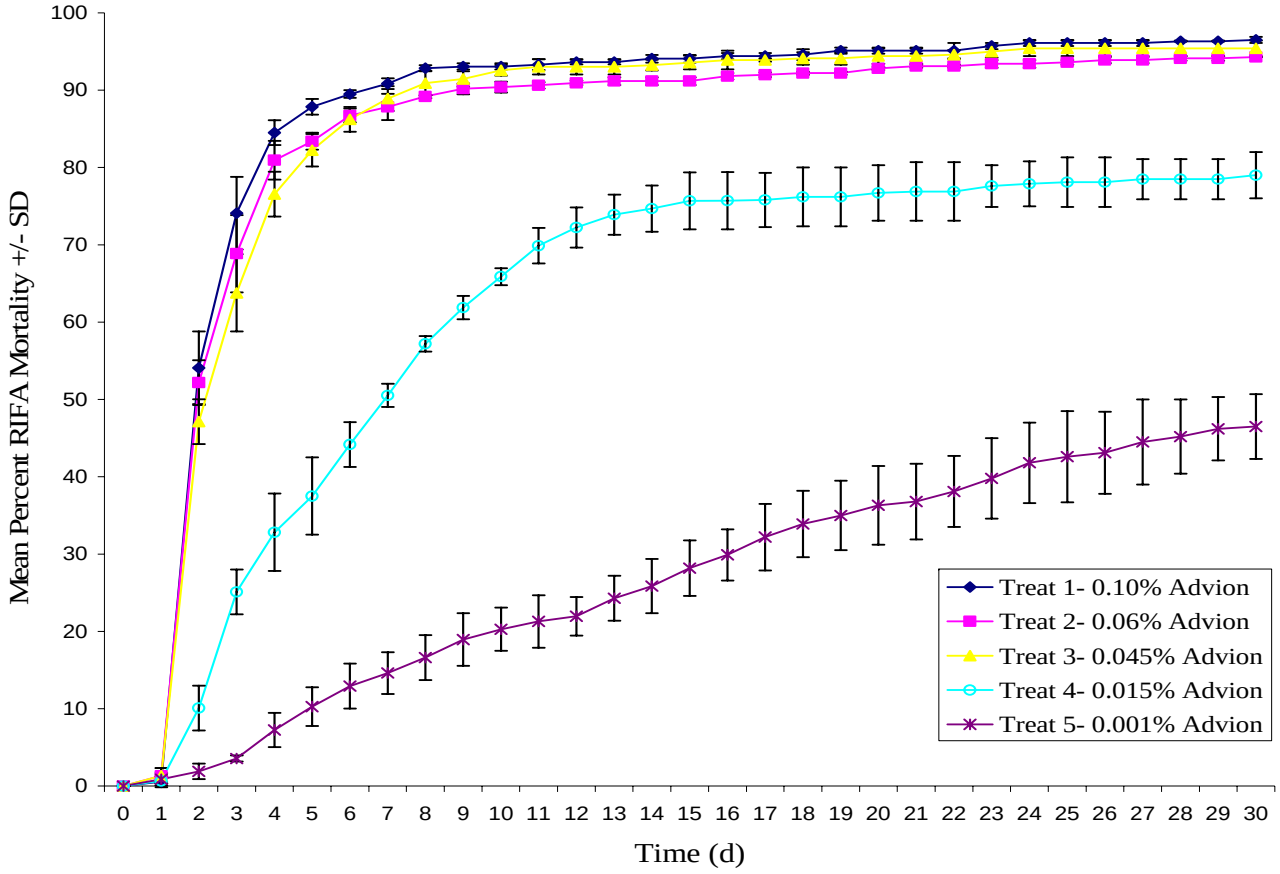

Figure 5. A comparison of the mean percent RIFA mortality resulting from treatment with 0.10%, 0.06%, 0.045%, 0.015% and 0.001% Advion™.

2 d (days 3 and 5) of the experiment. Though treatment with 0.10% Advion™ resulted in significantly greater ($P<0.05$) RIFA mortality than treatment with 0.045% Advion™ for four out of the first 5 d, from day 6 through the end of the experiment there was no significant difference ($P>0.05$) in mortality among the treatments consisting of 0.10%, 0.06%, or 0.045% Advion™. Furthermore, RIFA mortality in each of these three treatment groups was significantly greater ($P<0.05$) than mortality in the treatment groups consisting of 0.015% Advion™ and 0.001% Advion™ for every day of the experiment except day 1, which was the day that all treatments experienced minimal mortality. From day 2 through the end of the experiment, RIFA mortality resulting from treatment with 0.001% Advion™ was significantly less ($P<0.05$) than that resulting from any of the other treatments.

A comparison of the LT_{50}'s and LT_{90}'s among treatments is shown in Table 6. The LT_{50}'s for treatment with 0.10%, 0.06%, and 0.045% Advion™ were all slightly greater than 2 d, and the LT_{50}'s for all three treatments were separated by less than one half of a day. Similarly, the LT_{90}'s of those same three treatments were separated by less than 1 d, with all attaining LT_{90}'s of between 7 and 8 d. The LT_{50} for treatment with 0.015% Advion™ (LT_{50}=6.7 d) was considerably greater than that of the three previously mentioned treatments, and treatment with 0.001% Advion™ did not attain an LT_{50}. Neither treatment with 0.015% Advion™ or treatment with 0.001% Advion™ attained an LT_{90}.

Table 6. Comparison of the LT_{50}'s and the LT_{90}'s for RIFA that were treated with 0.10%, 0.06%, 0.045%, 0.015% and 0.001% Advion™.

	LT_{50} and LT_{90} per treatment (d)				
Lethal time	Treatment 1- 0.10% Advion™	Treatment 2- 0.06% Advion™	Treatment 3- 0.045% Advion™	Treatment 4- 0.015% Advion™	Treatment 5- 0.001% Advion™
LT_{50}	2.2	2.4	2.5	6.7	—[a]
LT_{90}	7.2	8.0	7.8	—[b]	—[b]

[a] An LT_{50} was not attained during this experiment.
[b] An LT_{90} was not attained during this experiment.

A comparison among treatments of RIFA feeding activity is shown in Table 7 ($F=5.23$; $df=209$; $P<0.05$). When comparing treatment with 0.10%, 0.06%, and 0.045% Advion™, there was no significant difference ($P>0.05$) in feeding activity for seven of the eight observation periods during which feeding occurred. However, RIFA feeding activity on 0.001% Advion™ and 0.0% Advion™ was significantly greater ($P<0.05$) than feeding activity on 0.10%, 0.06%, and 0.045% Advion™ for each of those eight observation periods. Feeding activity of RIFA on 0.015% Advion™ was significantly greater ($P<0.05$) than feeding activity on 0.10%, 0.06%, or 0.045% Advion™ for five out of the first eight observation periods, and significantly less ($P<0.05$) than feeding activity on both 0.001% and 0.0% Advion™ for five out of the first eight observation periods. Feeding activity on 0.06% and 0.045% Advion™ had ceased by the 48 h observation, and feeding activity on 0.10% Advion™ had ceased by the 96 h observation. However, feeding activity on 0.015%, 0.001% and 0.0% Advion™ continued well past that time, with feeding activity on 0.015% Advion™ eventually

Table 7. Comparison of feeding activity of the RIFA on 0.10%, 0.06%, 0.045%, 0.015%, 0.001%, and 0.0% Advion™.

Time (h)	Mean number of ants feeding in weigh dish per treatment[a] (mean ± SD)					
	Treatment 1- 0.10% Advion™	Treatment 2- 0.06% Advion™	Treatment 3- 0.045% Advion™	Treatment 4- 0.015% Advion™	Treatment 5- 0.001% Advion™	Control- 0.0% Advion (no indoxacarb)
1	29.3±6.7a	39.3±7.2b	38.3±5.1b	37.0±5.6ab	58.7±4.5c	56.0±3.6c
2	42.0±6.6a	42.3±3.2a	46.7±4.7a	46.3±6.8a	70.3±7.5b	77.0±4.6b
3	44.3±6.7a	40.3±3.5a	45.3±4.2a	59.3±5.1b	71.0±7.0c	71.7±4.0c
4	43.0±3.5a	38.3±2.5a	44.3±4.0a	63.0±7.0b	73.3±6.7c	68.0±1.7bc
5	41.7±3.8a	39.0±3.6a	44.0±2.0a	69.3±5.1b	74.3±5.5b	67.7±5.1b
24	36.3±3.8abc	28.7±7.8a	30.0±1.0a	31.7±1.5ab	41.7±6.5c	38.0±6.6bc
48	0.7±1.2a	0.7±1.2a	0.3±0.6a	26.7±5.8b	45.3±6.7c	40.3±5.1c
72	0.3±0.6a	0.0±0.0a	0.0±0.0a	23.7±1.2b	42.7±7.0c	38.0±4.4c
96	0.0±0.0a	0.0±0.0a	0.0±0.0a	18.7±3.2b	33.0±5.6c	35.0±2.6c
120	0.0±0.0a	0.0±0.0a	0.0±0.0a	20.0±5.6b	31.0±10.0c	32.7±1.2c
144	0.0±0.0a	0.0±0.0a	0.0±0.0a	10.3±0.6b	30.7±11.0c	33.3±3.2c
168	0.0±0.0a	0.0±0.0a	0.0±0.0a	10.3±0.6b	29.0±13.0c	24.7±3.1c
192	0.0±0.0a	0.0±0.0a	0.0±0.0a	7.3±4.0a	24.7±5.7b	31.0±6.6b
216	0.0±0.0a	0.0±0.0a	0.0±0.0a	5.0±6.1a	21.7±1.2b	27.7±3.5b
240	0.0±0.0a	0.0±0.0a	0.0±0.0a	2.0±1.7a	18.0±7.2b	23.3±1.5b
264	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	20.3±1.5b	22.7±1.5b
288	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	16.7±5.8b	18.3±7.2b
312	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	20.3±0.6b	23.3±2.1b
336	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	17.3±4.0b	16.3±4.9b
360	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	13.7±1.5b	21.3±2.9b
384	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	11.0±1.0b	18.7±4.9b
408	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	10.7±2.1b	19.0±6.1c
432	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	10.0±1.7b	16.3±7.1b
456	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	10.0±3.0b	12.3±2.5b
480	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	7.0±5.2ab	13.1±1.0b
504	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	4.0±6.1ab	11.0±3.6b
528	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	4.7±4.7ab	12.3±2.5b
552	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.3±0.6a	8.0±4.4b
576	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.7±1.2a	10.7±0.6b
600	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	7.3±6.0a
624	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	10.3±2.1b

[a] Means within a row with different letters are significantly different at P<0.05. Means were separated using the LSD test.

ceasing by hour 264, feeding activity on 0.001% Advion™ ceasing by hour 600, and feeding activity on 0.0% Advion™ continuing for the duration of the experiment. Feeding activity was greatest for all ants during the first 5 h of the experiment.

A comparison among treatments of the amount of bait removed from the weigh dishes is shown in Table 8 ($F=2.57$; $df=35$; $P<0.05$). There was no significant difference ($P>0.05$) in the amount of bait removed from the weigh dishes by the RIFA that were treated with 0.10%, 0.06%, 0.045% and 0.015% Advion™. Conversely, a significantly greater ($P<0.05$) amount of bait was removed from the weigh dishes by the RIFA thatwere treated with 0.001% Advion™ and 0.0% Advion™, with the greatest amount of bait being removed by the RIFA in the latter group. No bait was removed from the weigh dishes by the RIFA in any of the treatment groups or the control group following the first weighing event.

Grit Size. A comparison among treatments of the mean cumulative daily mortality is shown in Table 9 and Figure 6 ($F=2.27$; $df=89$; $P<0.05$). Treatment with only 0.025% Advion™ with standard sized grit resulted in significantly greater ($P<0.05$) RIFA mortality than did treatment with only 0.025% Advion™ with small sized grit for all but 1 d (day 1) of the experiment. Conversely, there was no significant difference ($P>0.05$) in RIFA mortality between treatment with only 0.025% Advion™ with standard sized grit and treatment with both 0.025% Advion™ with standard sized grit and 0.025% Advion™ with small sized grit for all but 4 d (days 5-8). There was no significant difference ($P>0.05$) in RIFA mortality between treatment with only 0.025% Advion™ with small sized grit and treatment with both 0.025% Advion™ with standard sized grit

Table 8. Weight-based comparison of the mean percentage of 0.10%, 0.06%, 0.045%, 0.015%, 0.001%, and 0.0% Advion™ that was removed from the weigh dish by the RIFA.

Day	Mean cumulative percent of bait removed from weigh dish per treatment[a] (mean ± SD)					
	Treatment 1- 0.10% Advion™	Treatment 2- 0.06% Advion™	Treatment 3- 0.045% Advion™	Treatment 4- 0.015% Advion™	Treatment 5- 0.001% Advion™	Control- 0.0% Advion™ (no indoxacarb)
5	10.0±0.0a	10.0±0.0a	13.3±5.8a	16.7±5.8a	50.0±10.0b	76.7±5.8c
10	10.0±0.0a	10.0±0.0a	13.3±5.8a	16.7±5.8a	50.0±10.0b	76.7±5.8c
15	10.0±0.0a	10.0±0.0a	13.3±5.8a	16.7±5.8a	50.0±10.0b	76.7±5.8c
20	10.0±0.0a	10.0±0.0a	13.3±5.8a	16.7±5.8a	50.0±10.0b	76.7±5.8c
25	10.0±0.0a	10.0±0.0a	13.3±5.8a	16.7±5.8a	50.0±10.0b	76.7±5.8c
30	10.0±0.0a	10.0±0.0a	13.3±5.8a	16.7±5.8a	50.0±10.0b	76.7±5.8c

[a] Means within a row with different letters are significantly different at $P<0.05$. Means were separated using the LSD test.

Table 9. Comparison of the mean percent RIFA mortality resulting from treatment with 0.025% Advion™ with standard sized grit (~2 mm/granule) and 0.025%Advion™ with small sized grit (<1 mm/granule).

Day	Mean cumulative percent mortality per treatment[a] (mean ± SD)		
	Treatment 1- 0.025% Advion™ with standard sized grit	Treatment 2- 0.025% Advion™ with small sized grit	Treatment 3- 0.025% Advion™ with standard sized grit and small sized grit
1	1.8±1.8a	0.0±0.0a	2.7±1.2a
2	40.8±3.1a	32.0±2.0b	40.5±2.9a
3	55.3±3.1a	49.7±4.0b	53.2±3.0ab
4	64.7±3.1a	59.7±1.8b	61.6±2.9ab
5	71.0±3.0a	66.4±2.0b	66.6±2.9b
6	79.4±3.1a	71.1±2.0b	72.3±1.2b
7	83.0±2.3a	76.1±2.0b	77.0±1.2b
8	85.4±3.1a	80.1±1.3b	81.3±1.0b
9	86.4±3.1a	81.0±2.6b	84.0±1.0ab
10	86.9±3.0a	81.9±1.9b	86.0±1.0ab
11	88.6±3.2a	81.9±2.7b	86.7±0.6a
12	90.3±2.7a	82.1±2.4b	87.0±0.6a
13	90.4±2.6a	82.1±2.4b	87.3±1.0a
14	91.1±2.8a	82.6±2.7b	88.0±0.6a
15	91.4±2.6a	83.2±2.0b	88.3±1.0a

[a] Mortality was corrected by the formula in Abbott (1925). Means within a row with different letters are significantly different at P<0.05. Means were separated using the LSD test.

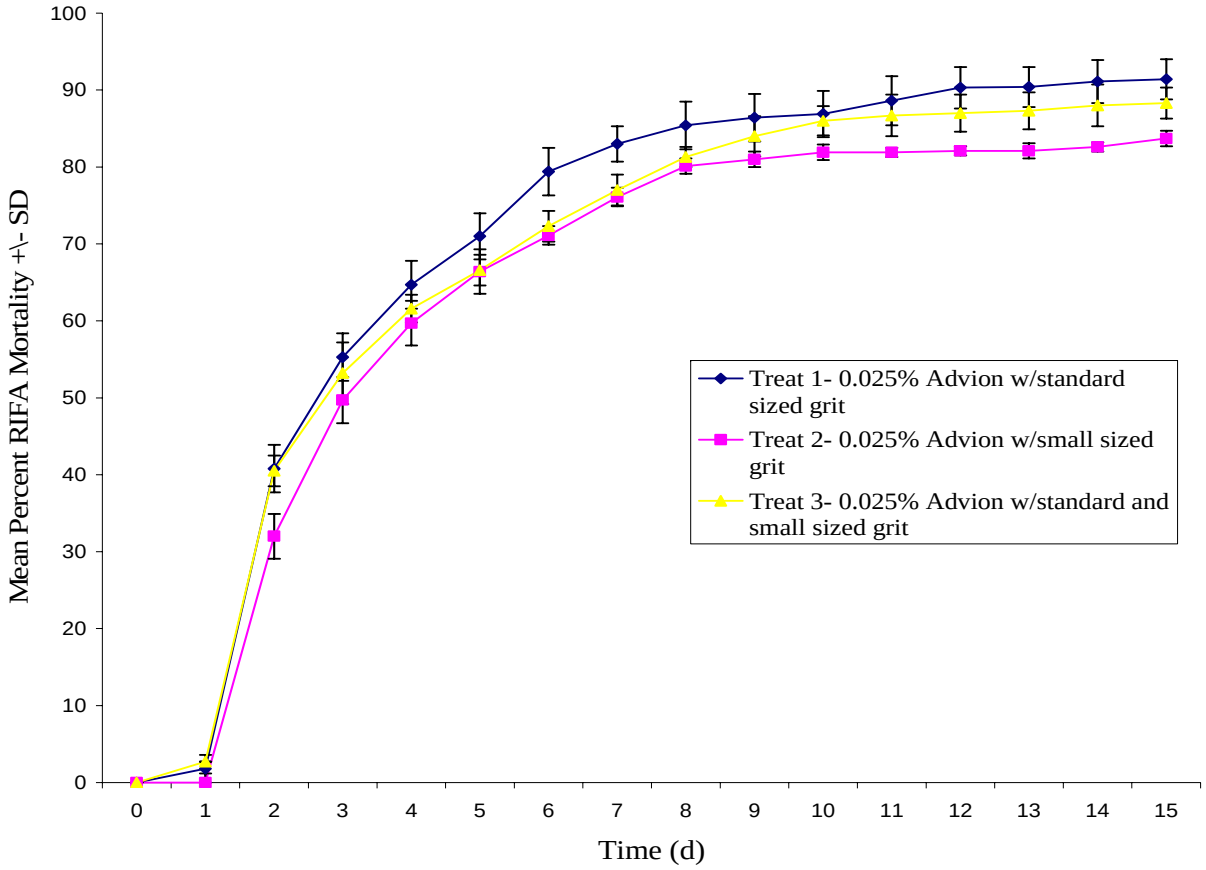

Figure 6. A comparison of the mean percent RIFA mortality resulting from treatment with 0.025% Advion™ with standard sized grit (~2 mm/granule) and 0.025%Advion™ with small sized grit (<1 mm/granule).

and 0.025% Advion™ with small sized grit for nine of the first 10 d, though the latter treatment resulted in significantly greater ($P<0.05$) mortality than the former from day 11 through the end of the experiment.

A comparison of the LT_{50}'s and LT_{90}'s among treatments is shown in Table 10. The LT_{50}'s of the three treatments ranged from 3.0-3.5 d, and only one half of a day separated them. There was, however, a 2 d difference between the LT_{90}'s of treatment with only 0.025% Advion™ with standard sized grit (LT_{90}=12.0 d), and treatment with both 0.025% Advion™ with standard sized grit and 0.025% Advion™ with small sized grit (LT_{90}=13.9 d). The difference between the LT_{90}'s of treatment with only 0.025% Advion™ with standard sized grit (LT_{90}=12.0 d) and treatment with only 0.025% Advion™ with small sized grit (LT_{90}=21.8 d) was ~10 d.

A comparison among treatments of RIFA feeding activity is shown in Table 11, Figure 7, and Figure 8 ($F=5.35$; $df=279$; $P<0.05$). When comparing treatment with only 0.025% Advion™ with standard sized grit and treatment with only 0.025% Advion™ with small sized grit, there was no significant difference ($P>0.05$) in feeding activity for the first six observation periods, nor was there any significant difference ($P>0.05$) in feeding activity between the two previously mentioned treatments and their corresponding control groups for the first six observation periods. However, feeding activity was significantly greater ($P<0.05$) in the treatment consisting of only 0.025% Advion™ with small sized grit than in the treatment consisting of only 0.025% Advion™ with standard sized grit for observation periods seven and eight, as feeding activity in the latter treatment had ceased by the 48 h observation and feeding activity in

Table 10. Comparison of the LT_{50}'s and the LT_{90}'s for RIFA that were treated with 0.025% Advion™ with standard sized grit (~2 mm/granule) and 0.025% Advion™ with small sized grit (<1 mm/granule).

	LT_{50} and LT_{90} per treatment (d)		
Lethal time	Treatment 1- 0.025% Advion™ with standard sized grit	Treatment 2- 0.025% Advion™ with small sized grit	Treatment 3- 0.025% Advion™ with standard sized grit and small sized grit
LT_{50}	3.0	3.5	3.2
LT_{90}	12.0	21.8	13.9

Table 11. Comparison of feeding activity of the RIFA on 0.025% Advion™ with standard sized grit (~2.0 mm/granule), 0.025% Advion™ with small sized grit (<1.0 mm/granule), 0.0% Advion™ (no indoxacarb) with standard sized grit, and 0.0% Advion™ (no indoxacarb) with small sized grit. Treatments 1 and 2, and Controls 1 and 2 were no-choice tests. Treatment 3 and Control 3 were choice tests.

	Mean number of ants feeding in weigh dish per treatment[a] (mean ± SD)							
Time (h)	Treatment 1- 0.025% Advion™ with standard sized grit	Treatment 2- 0.025% Advion™ with small sized grit	Treatment 3- 0.025% Advion™ with standard sized grit	Treatment 3- 0.025% Advion™ with small sized grit	Control 1- 0.0% Advion™ with standard sized grit	Control 2- 0.0% Advion™ with small sized grit	Control 3- 0.0% Advion™ with standard sized grit	Control 3- 0.0% Advion™ with small sized grit
1	36.3±2.5a	32.7±3.1ab	27.0±3.6b	28.7±3.2bc	35.0±5.0ac	34.3±7.1ac	31.7±7.1abc	34.3±4.0ac
2	40.0±7.8ab	39.7±4.5ab	30.0±8.0c	36.0±8.7bc	43.0±2.7ab	44.7±4.2a	43.3±5.8ac	43.3±2.3ab
3	47.0±4.6a	45.7±5.7a	28.7±3.2b	35.7±6.8b	50.7±6.4a	48.3±2.9a	46.7±8.2a	49.0±7.8a
4	50.7±7.6a	46.0±6.0a	29.0±3.0b	36.3±5.7b	48.0±7.9a	48.3±3.1a	49.3±1.5a	50.0±2.0a
5	44.3±5.7a	43.7±5.1a	30.3±3.6b	35.3±2.2b	49.0±3.0a	44.3±5.1a	44.0±3.6a	49.3±1.5a
24	37.3±6.7a	33.0±4.6a	10.7±1.2b	14.3±4.9b	39.0±4.0ac	37.3±7.6a	45.0±1.0c	45.7±3.1c
48	0.0±0.0a	32.7±5.5b	0.7±1.2a	0.0±0.0a	40.0±2.7c	36.0±1.7bc	42.7±4.6c	50.0±2.7d
72	0.0±0.0a	28.7±7.5b	0.3±0.6a	0.0±0.0a	33.7±3.1bc	31.3±3.2b	39.0±1.0cd	41.0±5.3d
96	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	29.7±4.5b	32.7±4.5bc	39.3±3.8cd	45.3±4.0d
120	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a	30.3±2.9b	30.3±7.1b	40.3±5.8c	44.7±5.0c

[a] Means within a row with different letters are significantly different at $P<0.05$. Means were separated using the LSD test.

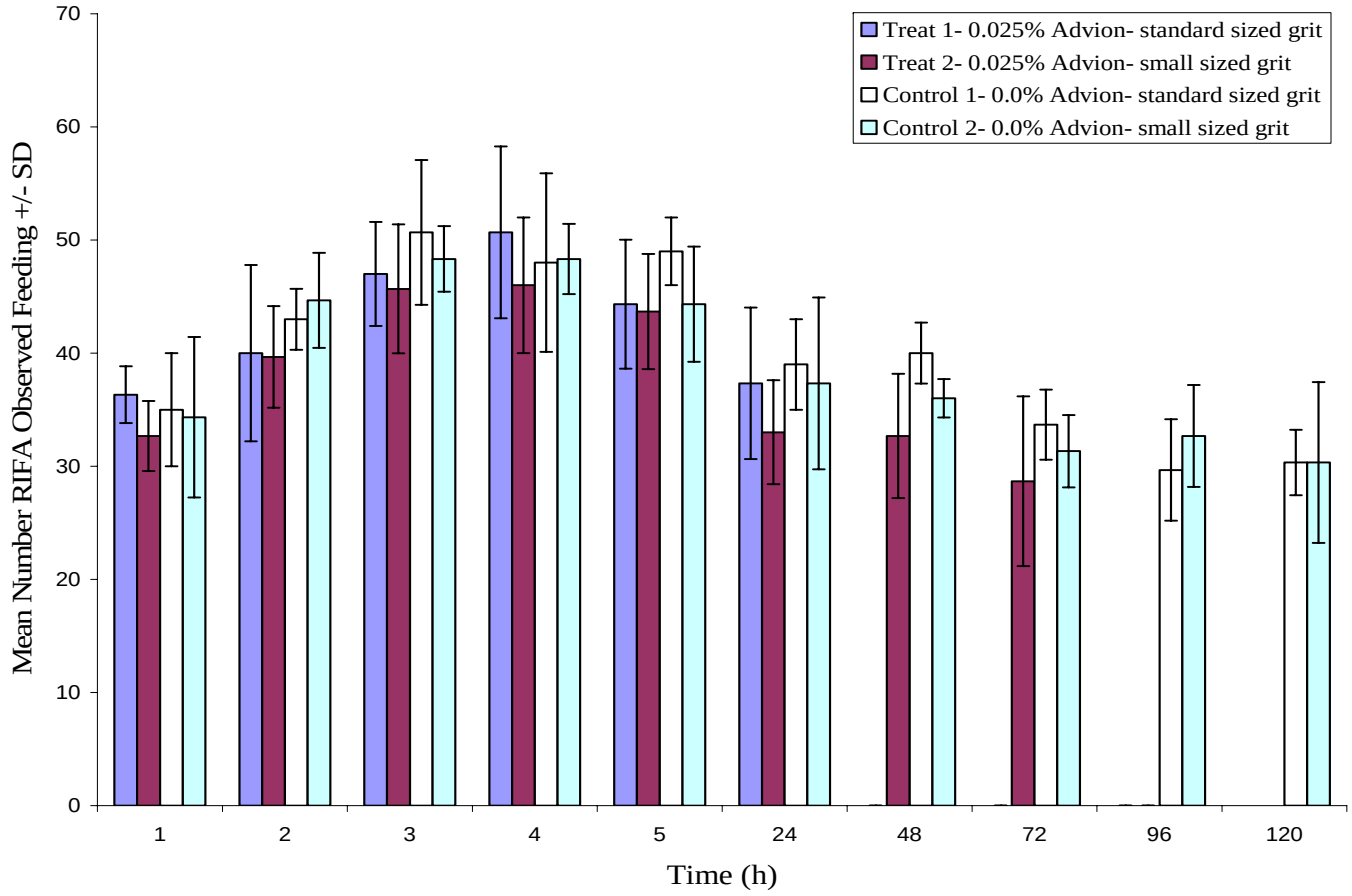

Figure 7. A comparison of feeding activity of the RIFA on 0.025% Advion™ with standard sized grit (~2 mm/granule), 0.025% Advion™ with small sized grit (<1 mm/granule), 0.0% Advion™ (no indoxacarb) with standard sized grit, and 0.0% Advion™ (no indoxacarb) with small sized grit. For each treatment, the ants were only given one size of grit to feed upon: either 1 g of standard sized grit or 1 g of small sized grit.

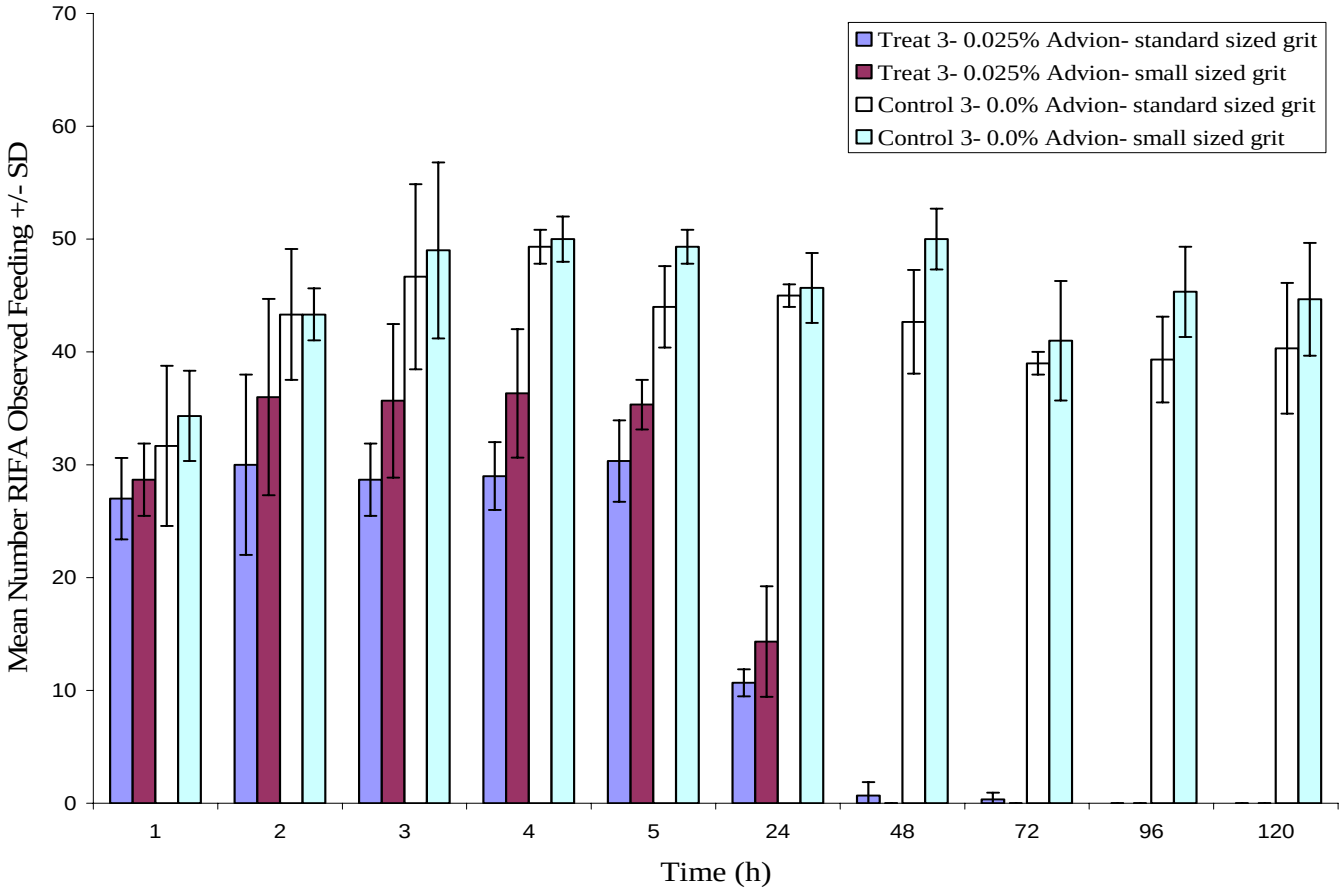

Figure 8. A comparison of feeding activity of the RIFA on 0.025% Advion™ with standard sized grit (~2 mm/granule), 0.025% Advion™ with small sized grit (<1 mm/granule), 0.0% Advion™ (no indoxacarb) with standard sized grit, and 0.0% Advion™ (no indoxacarb) with small sized grit. For Treatment 3 and Control 3, RIFA had a choice between the two sizes of grit, as they were supplied with 1 g of each in separate weigh dishes.

the former treatment did not cease until the 96 h observation. When comparing feeding activity on small sized grit and standard sized grit among the RIFA in the treatment consisting of both 0.025% Advion™ with standard sized grit and 0.025% Advion™ with small sized grit, there was no significant difference ($P>0.05$) for any of the observation periods, though the RIFA in the corresponding control group exhibited significantly greater ($P<0.05$) feeding activity on both sizes of grit for all but the first two observation periods. For this experiment, all feeding activity had ceased in each of the treatment groups by at least the 96 h observation, though feeding continued in each of the control groups for the duration of the experiment.

A comparison among treatments of the amount of bait removed from the weigh dishes is shown in Table 12 ($F=7.15$; $df=47$; $P<0.05$). There was no significant difference ($P>0.05$) in the amount of bait removed from the weigh dishes by the RIFA that were treated with only 0.025% Advion™ with standard sized grit, the RIFA that were treated with only 0.025% Advion™ with small sized grit, or the RIFA that were treated with both 0.025% Advion™ with standard sized grit and 0.025% Advion™ with small sized grit. However, when comparing each treatment group to its corresponding control group, in each instance the RIFA removed a significantly greater ($P<0.05$) amount of bait from the weigh dishes in the control groups than in the treatment groups. No bait was removed from the RIFA in the control groups after the second weighing event, while no bait was removed by the RIFA in the treatment groups after the first weighing event.

Table 12. Weight-based comparison of the mean percentage of 0.025% Advion™ with standard sized grit (~2 mm/granule), 0.025% Advion™ with small sized grit (<1 mm/granule), 0.0% Advion™ (no indoxacarb) with standard sized grit, and 0.0% Advion™ (no indoxacarb) with small sized grit that was removed from the weigh dish by the RIFA. Treatments 1 and 2, and Controls 1 and 2 were no-choice tests. Treatment 3 and Control 3 were choice tests.

Day	Mean cumulative percentage of bait removed from weigh dish per treatment[a] (mean ± SD)							
	Treatment 1- 0.025% Advion™ with standard sized grit	Treatment 2- 0.025% Advion™ with small sized grit	Treatment 3- 0.025% Advion™ with standard sized grit	Treatment 3- 0.025% Advion™ with small sized grit	Control 1- 0.0% Advion™ with standard sized grit	Control 2- 0.0% Advion™ with small sized grit	Control 3- 0.0% Advion™ with standard sized grit	Control 3- 0.0% Advion™ with small sized grit
5	13.3±5.8a	10.0±0.0a	16.7±5.8a	13.3±5.8a	76.7±5.8b	66.7±5.8c	63.3±5.8c	43.3±5.8d
10	13.3±5.8a	10.0±0.0a	16.7±5.8a	13.3±5.8a	80.0±0.0b	70.0±0.0c	76.7±5.8bc	50.0±5.8d
15	13.3±5.8a	10.0±0.0a	16.7±5.8a	13.3±5.8a	80.0±0.0b	70.0±0.0c	76.7±5.8bc	50.0±5.8d
20	13.3±5.8a	10.0±0.0a	16.7±5.8a	13.3±5.8a	80.0±0.0b	70.0±0.0c	76.7±5.8bc	50.0±5.8d
25	13.3±5.8a	10.0±0.0a	16.7±5.8a	13.3±5.8a	80.0±0.0b	70.0±0.0c	76.7±5.8bc	50.0±5.8d
30	13.3±5.8a	10.0±0.0a	16.7±5.8a	13.3±5.8a	80.0±0.0b	70.0±0.0c	76.7±5.8bc	50.0±5.8d

[a] Means within a row with different letters are significantly different at P<0.05. Means were separated using the LSD test.

Discussion and Conclusions

With little research on Advion™ having been conducted and published, it was necessary to examine some of the numerous technical aspects of this new RIFA bait. Data from this research documents the most effective chemical form and concentrations of indoxacarb, as well as the most appropriate grit size, for use in Advion™. First, in terms of the most effective chemical form of indoxacarb, the data found in Tables 1 and 2 revealed that treatment with only Advion™ containing JT333 resulted in significant RIFA mortality more quickly than did treatment with only Advion™ containing indoxacarb. In fact, for the RIFA treated with only Advion™ containing JT333, it only took ~1 d to reach 50% mortality, while it took 2.5 d to reach the same level of mortality for the RIFA that were treated with only Advion™ containing indoxacarb. However, the data also indicated that Advion™ containing JT333 worked too quickly, as mortality resulting from treatment with Advion™ containing JT333 had already reached a plateau at slightly over 70% by only the fourth day. If the active ingredient within a bait kills the foragers too quickly, then they do not have enough time to effectively share the toxin with other caste members, thus potentially weakening a colony but likely allowing it to survive (Su and Scheffrahn 1991). This is apparently what occurs when RIFA are treated with Advion™ containing JT333.

Though very rapid in comparison to most other RIFA baits, Advion™ containing indoxacarb works slowly enough to allow the foragers to distribute the insecticide throughout the colony, thus effectively killing the colony (Barr 2003; Wing et al. 2000). Despite taking more than twice as long as treatment with Advion™ containing JT333 to

achieve 50% mortality, treatment with Advion™ containing indoxacarb resulted in over 90% mortality by day 8, a level significantly greater ($P<0.05$) than that attained at any point in the experiment by treatment with Advion™ containing JT333. Treatment with both Advion™ containing indoxacarb and Advion™ containing JT333 resulted in a level of mortality similar to that resulting from treatment with only Advion™ containing JT333, which was significantly less ($P<0.05$) than the level of mortality resulting from treatment with only Advion™ containing indoxacarb. Resultantly, these data indicated that treatment with only Advion™ containing indoxacarb was the most effective treatment in this experiment with respect to RIFA mortality.

In terms of RIFA feeding preference, there was no significant difference ($P>0.05$) in feeding activity on Advion™ containing indoxacarb and on Advion™ containing JT333 among any of the three treatment groups. Similarly, there was no significant difference ($P>0.05$) in the amount of Advion™ containing indoxacarb and the amount of Advion™ containing JT333 that was removed from the weigh dishes among any of the treatment groups. This lack of a significant difference in overall feeding activity indicated that the primary difference between the two chemical forms was the level of mortality achieved. Therefore, since treatment with Advion™ containing indoxacarb resulted in a significantly greater ($P<0.05$) level of RIFA mortality, it was determined that Advion™ containing indoxacarb was more effective at controlling the RIFA than Advion™ containing JT333.

In terms of the most effective concentrations of indoxacarb for use in Advion™, the results of this research indicated that there were three very effective concentrations:

0.10%, 0.06%, and 0.045%. As illustrated in Tables 5 and 6, treatment with each of these three concentrations resulted in 90% RIFA mortality by day 8 or 9 of the experiment, the LT_{50}'s of these concentrations were separated by less than one half of a day, the LT_{90}'s were separated by less than 1 d, and there was no significant difference ($P>0.05$) in mortality resulting from these three treatments after the fifth day of the experiment. Therefore, 0.10%, 0.06%, and 0.045% Advion™ were all considered to be very effective concentrations, with each ultimately achieving approximately the same level of RIFA control in approximately the same amount of time. The other two concentrations evaluated in this experiment, 0.015% and 0.001% Advion™, resulted in significantly less ($P<0.05$) RIFA mortality than the three previously mentioned concentrations, though treatment with 0.015% Advion™ did ultimately achieve 79% mortality. However, neither of these concentrations ever attained an LT_{90}, and 0.001% Advion™ never even attained an LT_{50}. Based on these data, it was determined that out of the five concentrations tested, 0.10%, 0.06%, and 0.045% Advion™ were the only concentrations that proved to be very effective at controlling the RIFA.

There was no significant difference ($P>0.05$) in feeding activity among the three very effective concentrations of Advion™, nor was there any significant difference ($P>0.05$) in the amount of bait removed from the weigh dishes by the RIFA that were treated with those concentrations. Therefore, it was determined that there was no significant difference ($P>0.05$) in overall feeding activity among the three treatments. Essentially, 0.10%, 0.06%, and 0.045% Advion™ proved to be equally effective in this experiment, since there was no significant difference in feeding activity among the three, and each

ultimately provided approximately the same amount of control in approximately the same amount of time. These results indicate that there is likely a threshold of effectiveness at ~0.045% active ingredient.

As is well known, when attempting to control pest insects with chemicals, it is always important to use the least amount of chemical that will still ultimately provide control of the selected pest (Pedigo 2002). In this experiment, out of the three very effective concentrations, 0.045% Advion™ contained the lowest concentration of indoxacarb. Resultantly, though 0.10%, 0.06%, and 0.045% Advion™ all provided very effective control of the RIFA, out of all concentrations of indoxacarb that were tested in the experiment it was determined that 0.045% was the most appropriate concentration of indoxacarb for use in Advion™.

With relation to grit size, the data found in Tables 9 and 10 indicated that treatment with only 0.025% Advion™ with standard sized grit resulted in significantly greater RIFA mortality ($P<0.05$) than treatment with only 0.025% Advion™ with small sized grit. The RIFA that were treated with both 0.025% Advion™ with standard sized grit and 0.025% Advion™ with small sized grit experienced mortality levels similar to the RIFA that were treated with only 0.025% Advion™ with standard sized grit. However, the LT_{90} for the RIFA that were treated with both sizes of grit was 2 d longer in duration than that of the RIFA that were treated with only standard sized grit. Therefore, in terms of the level of RIFA mortality and the speed at which that level of mortality was achieved, treatment with only 0.025% Advion™ with standard sized grit was the most effective treatment in this experiment. This is possibly due to the fact that it is

considerably easier, less time consuming, and less energy consuming for a RIFA worker to forage for and feed upon a single standard sized granule than many small sized granules. In order for a single worker to consume the same quantity of oil (and, thus, indoxacarb solubilized in the oil) that could be acquired from a single standard sized granule, the worker would have to spend a considerably greater amount of time foraging for and feeding upon many small sized granules.

When comparing treatment with only 0.025% Advion™ with standard sized grit to treatment with only 0.025% Advion™ with small sized grit in terms of feeding preference, the data in Table 11 indicated that there was no significant difference ($P>0.05$) in feeding activity for the first six observation periods. Though feeding on the small sized grit continued for 2 d longer than it did on standard sized grit, this was not indicative of preference. Most likely, this was due to the simple fact that more foragers were still alive and capable of feeding in the treatment consisting of small sized grit, since that treatment resulted in significantly less ($P<0.05$) mortality than did treatment with standard sized grit. In the treatment consisting of both small sized grit and standard sized grit, there was no significant difference ($P>0.05$) in feeding activity for any observation period. Furthermore, there was no significant difference ($P>0.05$) in the amount of bait removed from the weigh dishes by the RIFA among any of the three treatment groups. Therefore, it was determined that the RIFA did not exhibit a preference for either of the grit sizes that were tested in this experiment. This, paired with the fact that significantly greater mortality ($P<0.05$) resulted from treatment with only 0.025% Advion™ with standard sized grit than treatment with only 0.025%

Advion™ with small sized grit, indicated that the standard sized grit was more appropriate than the small sized grit size for use in Advion™.

In conclusion, the results of these experiments indicated that Advion™ containing indoxacarb was more effective at controlling the RIFA than Advion™ containing JT333, 0.045% was the most appropriate concentration of indoxacarb for use in Advion™ out of the three most effective concentrations (0.10%, 0.06%, and 0.045%) that were tested, and standard sized grit was more appropriate than small sized grit for use in Advion™. It should also be noted that for all three experiments the RIFA in the control groups exhibited significantly greater ($P<0.05$) overall feeding activity than did the RIFA that were treated with any concentration of Advion™ above 0.025%. Beginning with 0.025% Advion™, the lower the concentration, the more similar the feeding activity was to that exhibited in the control groups. This appeared to indicate that the RIFA were more sensitive to indoxacarb at levels above 0.025%.

Future experimentation should examine different concentrations of Advion™ other than the five that were evaluated in this study, focusing on those concentrations between 0.015% and 0.045%. Additionally, different grit sizes should be evaluated, primarily those varying in size between the standard sized grit and the small sized grit used in this study. Furthermore, Advion™ containing concentrations of JT333 that are less than the 0.045% concentration used in this study should be utilized and evaluated to determine if a lower concentration of the metabolite would be more effective.

CHAPTER III

DETERMINATION OF THE MOST EFFECTIVE CONCENTRATION AND QUANTITY OF ADVION™, AS WELL AS THE MOST EFFECTIVE PLACEMENT OF THE BAIT, FOR INDIVIDUAL *Solenopsis invicta* BUREN MOUND TREATMENTS

Introduction

Advion™ (E.I. DuPont de Nemours and Company, Wilmington, DE) is a new corncob grit-based bait that contains the active ingredient indoxacarb, which is a pyrazoline-type insecticide classified as an oxadiazine. Discovered by E.I. DuPont de Nemours and Company in 1991, the chemical indoxacarb is a novel insecticide that must first be metabolized in order to become acutely toxic. Following ingestion by the insect, metabolic breakdown of indoxacarb occurs via amidase and esterase enzymes commonly found within the midgut and/or fat bodies, ultimately producing an N-decarbomethoxyllated metabolite known as JT333. It is JT333 that is toxic to the insect, functioning as a potent, voltage-dependent sodium channel blocker. The bio-activation of this potent metabolite within the insect ultimately results in severe neurotoxic symptoms, paralysis, and a relatively rapid death (Wing et al. 2000).

Receiving approval from the United States Environmental Protection Agency in August of 2004 (EPA Registration #352-627), indoxacarb-containing Advion™ was developed for the control of the red imported fire ant (RIFA), *Solenopsis invicta* Buren.

As with many RIFA baits, Advion™ can either be applied as a broadcast treatment or utilized as an individual mound treatment. While both methods have the potential to effectively achieve control of the RIFA, individual mound treatments are generally more expensive and more labor intensive than broadcast treatments due to the fact that a greater amount of bait is usually necessary to attain equivalent control, and the fact that each mound in an area must be treated individually. Research has shown that individual mound treatments often achieve control more rapidly than broadcast treatments. Furthermore, individual mound treatments are necessary and more practical in certain situations, such as scenarios in which more immediate control is required, or when only a small area needs to be treated. Nevertheless, in the majority of cases, the most effective form of RIFA control is to utilize some combination of both broadcast treatments and individual mound treatments (Barr 1999; Barr and Best 1999; Barr et al. 1999; Drees et al. 1996; Vail 1998).

Limited research on broadcast treatments with Advion™ has been conducted and published (Barr 2003). However, no research has been conducted and published with relation to individual RIFA mound treatments with Advion™. Therefore, the focus of this research was individual RIFA mound treatments with Advion™ and, specifically, determination of the most effective concentration and quantity of Advion™, as well as the most effective placement of the bait, for individual RIFA mound treatments. In terms of concentrations and quantities, this research entailed numerous individual RIFA mound treatments with multiple concentrations and quantities of Advion™ in an attempt

to determine the most effective concentration and quantity of Advion™ for controlling individual RIFA mounds.

With relation to the most effective placement of Advion™, there is some debate over what is the appropriate distance from a mound that bait should be applied for individual mound treatments. It is not uncommon for different bait labels to recommend placing the bait anywhere from directly on top of the mound to up to 1 m away from the mound (Apperson et al. 1984; Gold et al. 1996a). Therefore, this research attempted to determine the most appropriate distance from the mound for bait placement, thus maximizing the effectiveness of Advion™ in terms of controlling individual RIFA mounds. Summarily, the objective of this research was to determine the most effective concentration and quantity of Advion™, as well as the most effective placement of the bait, for obtaining maximum control of RIFA colonies via individual mound treatments with Advion™.

Materials and Methods

The following two experiments were conducted in the field on the grounds of the privately owned Waterland Kennels (N29°59’83” W95°58’36”) in Waller County, Texas. The property was ~6 ha in size, with the front 2 ha consisting primarily of shortly mowed Bermuda grass and small ponds. This portion of the property housed the kennel. The back 4 ha of the property consisted of un-grazed, grass pastureland that was routinely mowed. Only the back 4 ha of the property were utilized for these experiments, as that area was not disturbed by the dogs residing at the kennel. The grass

pastureland was mowed ~1 wk prior to beginning an experiment, and then was not mowed again for the duration of the experiment. All of the Advion™ used in the two experiments that were conducted on this property was freshly produced and shipped directly from E.I. DuPont de Nemours and Company's research laboratory in Newark, Delaware.

Concentrations and Quantities. Experimentation comparing the efficacy of various concentrations and quantities of Advion™ against the RIFA was conducted for a total of 14 d (July 15-July 29, 2004). Three different concentrations of Advion™ were used for this experiment: 0.045% Advion™, 0.016% Advion™ and 0.008% Advion™. Concentrations were selected based on previous research conducted by Furman (2006) which compared the efficacy of treatment with various concentrations of Advion™ against the RIFA in a laboratory setting. For each of the three concentrations that were used in this experiment, four different quantities were tested: 5 g (1 Tbsp), 10 g (2 Tbsp), 15 g (3 Tbsp) and 20 g (4 Tbsp). As is shown in Table 13, a total of 12 different treatment groups, as well as an untreated control group, were utilized for this experiment.

There were three replicates for each of the treatment groups and the control group, with each replicate consisting of five mounds. Prior to beginning the experiment, mounds were selected by placing an individually numbered, 50 cm tall, metal wire, fluorescent colored flag in the center of a given mound. Once placed in the mound, the metal wire was vibrated to elicit a response from the RIFA and determine the activity level of the colony. The following Lichert scale was used to determine the activity level:

Table 13. Treatments administered in an attempt to determine the most effective concentrations and quantities of Advion™ for use with individual RIFA mound treatments.

Treatment	Concentration of Advion™ (%)	Quantity of Advion™ (g/mound)
1	0.045	5
2	0.045	10
3	0.045	15
4	0.045	20
5	0.016	5
6	0.016	10
7	0.016	15
8	0.016	20
9	0.008	5
10	0.008	10
11	0.008	15
12	0.008	20
control	—	—

"0" = inactive (no ants responding), "1" = minor activity (1-50 ants responding), "2" = moderate activity (51-100 ants responding), and "3" = fully active (more than 100 ants responding). Only colonies with a Lichert scale rating of "3" were selected for use in this experiment (Gold et al. 1996a, 1996b). In this manner, 39 groups of five mounds each were selected, with each group comprising a single plot. Each of the 39 plots was separated from the nearest plot by a distance of at least 10 m, and this distance was ensured by using a Rolatape® M300 series measuring wheel (Rolatape Corp., Spokane, WA).

On the morning of the first day of the experiment, a plastic tablespoon was used to apply the appropriate concentration and amount of Advion™ directly around the base of each of the mounds within each treatment group. Treatments were administered

between approximately 7:00 a.m. and 9:00 a.m. C.S.T. As previously mentioned, the mounds in the control group were left untreated. The next morning, ~ 24 h following treatment, the metal wire of the flag that had been placed in each mound was vibrated to elicit a response from the RIFA and measure the activity level of the colony. The response by the ants was graded according to the Lichert scale previously described, and colony mortality was assumed when a response of "0" was observed. The experiment was continued in this manner each day for the first 7 d, with one final reading for each colony being taken on day 14 of the experiment, thus ultimately producing a data set of colony mortality for each treatment. Additionally, the soil temperature and precipitation were measured. Soil temperature was measured with a Sergeant-Welch 12.7 cm soil thermometer (Sergeant-Welch, Buffalo Grove, IL) each morning within each of the plots for the first 7 d of the experiment, and then again on the last day of experimentation. Precipitation was measured with a Garden Treasures® 15.2 cm capacity rain gauge (Lowe's Companies Inc., Wilkesboro, NC) for each 24 h period for the first 7 d of the experiment. No 24 h precipitation readings were taken after the first 7 d.

Bait Placement. Experimentation comparing the effectiveness of individual RIFA mound treatments with Advion™ when the bait was placed at varying distances from the mound was conducted for a total of 14 d (August 5-August 19, 2004). For this experiment, 10 g (2 Tbsp) of 0.045% Advion™ were used to treat each RIFA mound, with that particular quantity and concentration being chosen based upon the results of the previous experiment. Therefore, since all RIFA mounds were treated with the same quantity and concentration of Advion™ in this experiment, the only variable among the

different treatments was the distance from the mound that the bait was placed. Advion™ was placed at four different distances from RIFA mounds, so there were a total of four different treatment groups and a control group for this experiment. Treatment 1 consisted of bait placement 0.5 m from the mound, Treatment 2 consisted of bait placement 1 m from the mound, Treatment 3 consisted of bait placement 2 m from the mound, Treatment 4 consisted of bait placement 3 m from the mound, and the RIFA mounds in the control group were left untreated. There were three replicates for each of the treatment groups and the control group, with each replicate consisting of seven mounds. Prior to beginning the experiment, mounds were selected and flagged, and colony activity was determined in a manner identical to that described in the previous experiment. In this manner, 15 groups of seven mounds each were selected, with each group comprising a single plot.

Once the mounds had been selected, then it was necessary to use flags and twine to form a circular border around each of the mounds that were used in the treatment groups, with the mound being centrally located within the circle (Figure 9). For Treatment 1, six flags were first placed in a 0.5 m radius circle around each mound, and then a single piece of #16 polyester/cotton twine was used to connect the six flags, thus forming a circular border. The same procedure was followed for the other three treatments, with the number of flags used per mound varying as follows: six flags were placed in a 1 m radius circle around each mound for Treatment 2, eight flags were placed in a 2 m radius circle around each mound for Treatment 3, and 10 flags were placed in a 3 m radius circle around each mound for Treatment 4. All distances from the center of each mound

Figure 9. The configuration for a typical mound in one of the treatment groups of the experiment that was conducted in order to compare the effectiveness of individual mound treatments with Advion™ when the bait was placed at varying distances from the mound. This was the configuration for mounds in Treatment 3, consisting of eight flags in a 2 m radius circle around the centrally located mound, and a circular border demarcated by a single piece of string connected to each of the eight flags.

to each flag that was placed in a circle around the mound were carefully measured with a Rolatape® M300 series measuring wheel (Rolatape Corp., Spokane, WA). Additionally, the measuring wheel was used to ensure that each of the 15 plots was separated from the nearest plot by at least 10 m.

On the first morning of the experiment, a plastic tablespoon was used to administer 2 Tbsp of 0.045% Advion™ to each of the mounds in the treatments groups. Treatments were administered between approximately 7:00 a.m. and 9:00 a.m.C.S.T. The bait was

evenly distributed in a circle around each mound, using the circular border formed by the string attached to the flags around each mound as a guide. As aforementioned, the mounds in the control group were left untreated. After 24 h, the metal wire of the flag within each mound was vibrated to elicit a response from the RIFA and measure the activity level of the colony in a manner identical to that described in the previous experiment. The experiment was continued in this manner each day for 7 d, with one final reading for each colony being taken on day 14 of the experiment, thus ultimately producing a data set of colony mortality for each treatment. Additionally, the soil temperature and precipitation were measured in a manner identical to that described in the previous experiment.

Statistics. At the conclusion of each experiment, SPSS® software (SPSS 2001) was used to conduct statistical analysis of the data. First, however, Abbott's formula was used to correct all mortality data (Abbott 1925). Abbott's formula for correcting mortality data is

$$[(X-Y) / X] \times 100 = \text{percent control}$$

where "X" is the percent colony survival in the control group, and "Y" is the percent colony survival in the treatment group. Statistical analysis was conducted with ANOVA on the corrected mortality data sets for both experiments. Finally, the LSD post hoc test was conducted on those same data sets to determine significant differences among treatments. All tests of significance were evaluated at $P = 0.05$.

Results

Concentrations and Quantities. The mean morning soil temperature and the daily precipitation are shown in Table 14. The mean morning soil temperature was always greater than 26.0° C, and no precipitation occurred within 2 d of when Advion™ was applied to the treatment groups. A comparison among treatments of the mean cumulative daily colony mortality is shown in Table 15 ($F=6.28$; $df=95$; $P<0.05$). For days 3-14, there was no significant difference ($P>0.05$) in colony mortality among treatments with 10 g, 15 g, or 20 g of 0.045% Advion™, and these three treatments resulted in significantly greater ($P<0.05$) colony mortality than any of the other treatments over that same time period. Further, the same three aforementioned treatments resulted in greater than 90% colony mortality by day 6 of the experiment. By day 7, there was no significant difference ($P>0.05$) in colony mortality among treatments with 10 g, 15 g, or 20 g of 0.045% Advion™, 20 g of 0.016% Advion™, or 20 g of 0.008% Advion™, and each of these treatments resulted in greater than 90% colony mortality. These five treatments had also all resulted in significantly greater ($P<0.05$) colony mortality by day 7 than any of the other treatments. By day 14, there was no significant difference ($P>0.05$) in colony mortality among treatments with 5 g, 10 g, 15g, or 20 g of 0.045% Advion™, 15 g or 20 g of 0.016% Advion™, or 15 g or 20g of 0.008% Advion™. These eight treatments resulted in significantly greater ($P<0.05$) colony mortality by day 14 than any of the remaining treatments.

A comparison of the LT_{50}'s and LT_{90}'s among treatments is shown in Table 16. The LT_{50}'s of shortest duration occurred among the treatments consisting of 10 g, 15 g, and

Table 14. Mean morning soil temperature and daily precipitation for the experiment that consisted of individual RIFA mound treatments with 5-20 g (1-4 Tbsp) of 0.045% Advion™, 0.016% Advion™, and 0.008% Advion™.

Day	Mean morning soil temperature ± SD (ºC)	Daily precipitation (cm)
0	26.8±0.6	0.0
1	27.4±0.6	0.0
2	27.1±0.5	0.0
3	26.6±0.5	1.0
4	26.1±0.4	0.3
5	26.3±0.6	<0.3
6	26.2±0.5	2.0
7	26.7±0.7	1.0
14	27.6±0.9	—[a]

[a] A precipitation reading was not taken on this day.

Table 15. Comparison of the mean percent RIFA colony mortality resulting from treatments consisting of 5-20 g (1-4 Tbsp) of 0.045% Advion™, 0.016% Advion™, and 0.008% Advion™.

	Mean cumulative percent colony mortality[a] (mean ± SD)							
Treatment	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 14
5 g 0.045%	0.0±0.0a	13.3±11.6a	33.3±11.6a	46.7±11.6ad	66.7±11.6a	71.4±10.3af	78.1±3.3a	98.1±3.3a
10 g 0.045%	0.0±0.0a	40.0±0.0bc	66.7±11.6b	86.7±11.6b	86.7±11.6b	96.2±3.3b	96.2±3.3b	96.2±3.3a
15 g 0.045%	0.0±0.0a	46.7±11.6b	66.7±11.6b	86.7±11.6b	86.7±11.6b	91.4±10.3b	98.1±3.3b	98.1±3.3a
20 g 0.045%	0.0±0.0a	33.3±11.6c	66.7±11.6b	86.7±11.6b	86.7±11.6b	91.4±10.3b	98.1±3.3b	98.1±3.3a
5 g 0.016%	0.0±0.0a	0.0±0.0d	0.0±0.0c	13.3±11.6ce	20.0±0.0c	34.3±0.0ce	41.0±11.6c	81.0±11.6b
10 g 0.016%	0.0±0.0a	0.0±0.0d	6.7±11.6c	20.0±0.0c	46.7±11.6d	56.2±3.3d	76.2±3.3a	82.9±10.3b
15 g 0.016%	0.0±0.0a	0.0±0.0d	20.0±0.0d	40.0±0.0a	46.7±11.6d	56.2±3.3d	69.5±8.2a	96.2±3.3a
20 g 0.016%	0.0±0.0a	33.3±11.6c	53.3±11.6e	53.3±11.6d	66.7±11.6a	71.4±10.3af	91.4±10.3b	98.1±3.3a
5 g 0.008%	0.0±0.0a	0.0±0.0d	0.0±0.0c	6.7±11.6e	26.7±11.6c	26.7±11.6c	40.0±0.0c	60.0±0.0c
10 g 0.008%	0.0±0.0a	0.0±0.0d	6.7±11.6c	20.0±0.0c	26.7±11.6c	41.0±11.6e	54.3±0.0d	81.0±11.6b
15 g 0.008%	0.0±0.0a	0.0±0.0d	13.3±11.6cd	46.7±11.6ad	66.7±11.6a	76.2±3.3a	76.2±3.3a	89.5±8.2ab
20 g 0.008%	0.0±0.0a	0.0±0.0d	26.7±11.6ad	40.0±0.0a	46.7±11.6d	63.3±11.1df	90.0±8.7b	96.7±3.0a

[a] Mortality was corrected by the formula in Abbott (1925). Means within a column with different letters are significantly different at P<0.05. Means were separated using the LSD test.

Table 16. Comparison of the LT_{50}'s and the LT_{90}'s for RIFA colonies that were treated with 5-20 g (1-4 Tbsp) of 0.045% Advion™, 0.016% Advion™, and 0.008% Advion™.

Treatment	Lethal time (d)	
	LT_{50}	LT_{90}
Treatment 1- 5 g 0.045%	4.1	9.0
Treatment 2- 10 g 0.045%	2.5	5.2
Treatment 3- 15 g 0.045%	2.4	5.1
Treatment 4- 20 g 0.045%	2.6	5.1
Treatment 5- 5 g 0.016%	8.0	—[a]
Treatment 6- 10 g 0.016%	5.9	—[a]
Treatment 7- 15 g 0.016%	5.2	10.5
Treatment 8- 20 g 0.016%	3.3	8.4
Treatment 9- 5 g 0.008%	9.8	—[a]
Treatment 10- 10 g 0.008%	7.2	—[a]
Treatment 11- 15 g 0.008%	4.7	—[a]
Treatment 12- 20 g 0.008%	4.7	8.8

[a] An LT_{90} was not attained during this experiment.

20 g of 0.045% Advion™. The LT_{50} for each of these treatments was less than 3 d, with less than one half of a day separating the three. The LT_{50}'s for all other treatments were greater than 4 d, with the exception of the treatment consisting of 20 g of 0.016% Advion™, which had an LT_{50} of 3.3 d. Further, the LT_{90}'s for the treatments consisting of 10 g, 15 g, and 20 g of 0.045% Advion™ were all virtually identical, with each being slightly greater than 5 d. The treatments consisting of 5 g of 0.045% Advion™, 15 g and 20 g of 0.016% Advion™, and 20 g of 0.008% Advion™ all attained LT_{90}'s, as well, with times ranging from 8.4 d to 10.5 d. None of the other five treatments attained an LT_{90} over the duration of this experiment.

Bait Placement. The mean morning soil temperature and the daily precipitation are shown in Table 17. The mean morning soil temperature was always greater than 26.0° C, and no precipitation occurred within 3 d of when Advion™ was applied to the treatment groups. A comparison among treatments of the mean cumulative daily colony mortality is shown in Table 18 and Figure 10 ($F=6.28$; $df=95$; $P<0.05$). Treatment consisting of bait placement 1 m from the mound resulted in significantly greater mortality ($P<0.05$) than the other treatments for days 2 and 3. During that same time period, treatment consisting of bait placement 3 m radius from the mound resulted in significantly less mortality ($P<0.05$) than the other treatments. By day 4, there were no significant differences ($P>0.05$) in colony mortality among treatments based on bait placement 0.5-2.0 m from the mound, though the colony mortality achieved by these three treatments was still significantly greater ($P<0.05$) than that achieved by treatment consisting of bait placement 3 m from the mound. Day 5 was the first day that any of

Table 17. Mean morning soil temperature and daily precipitation for the experiment that consisted of individual RIFA mound treatments with 10 g (2 Tbsp) of 0.045% Advion™ which were placed around mounds in circles of varying radii.

Day	Mean morning soil temperature ± SD (ºC)	Daily precipitation (cm)
0	26.8±0.7	0.0
1	27.1±0.5	0.0
2	27.4±0.4	0.0
3	27.8±0.8	0.0
4	27.6±0.4	0.3
5	27.7±0.5	0.0
6	27.4±0.6	0.0
7	27.0±0.7	0.0
14	27.9±0.8	—[a]

[a] A precipitation reading was not taken on this day.

Table 18. Comparison of the mean percent RIFA colony mortality resulting from treatments consisting of the placement of 10 g (2 Tbsp) of 0.045% Advion™ around a RIFA mound in a circle with a radius of 0.5 m, 1 m, 2 m, or 3 m.

Day	Mean cumulative percent colony mortality per treatment[a] (mean ± SD)			
	Treatment 1- Advion™ 0.5 m from mound	Treatment 2- Advion™ 1 m from mound	Treatment 3- Advion™ 2 m from mound	Treatment 4- Advion™ 3 m from mound
1	0.0±0.0a	0.0±0.0a	0.0±0.0a	0.0±0.0a
2	19.1±8.2a	33.3±8.3b	19.0±8.3a	4.8±8.3c
3	59.0±8.9a	63.3±8.5b	59.0±8.9a	42.9±7.8c
4	73.3±8.9a	77.6±8.5a	78.1±8.3a	61.9±8.3b
5	87.6±8.3a	87.1±8.1a	87.6±7.6a	90.5±8.3a
6	92.4±7.7a	96.7±0.4a	92.4±8.3a	90.5±8.3a
7	97.1±0.7a	96.7±0.4a	92.4±8.3a	90.5±8.3a
14	97.1±0.7a	96.7±0.4a	95.7±2.6a	93.8±2.5a

[a] Mortality was corrected by the formula in Abbott (1925). Means within a row with different letters are significantly different at $P<0.05$. Means were separated using the LSD test.

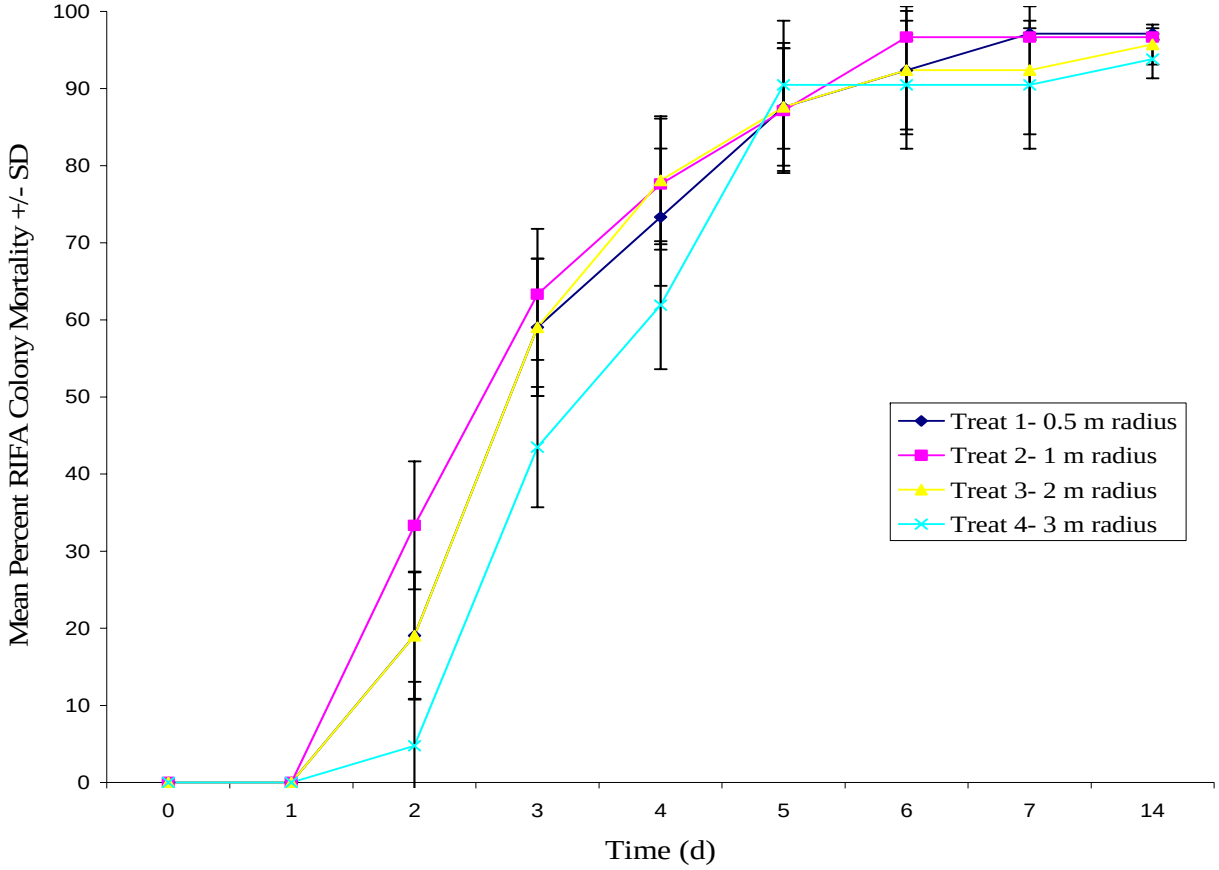

Figure 10. A comparison of the mean percent RIFA colony mortality resulting from treatments consisting of the placement of 10 g (2 Tbsp) of 0.045% Advion™ around a RIFA mound in a circle with a radius of 0.5 m, 1 m, 2 m, or 3 m.

the treatments had resulted in 90% colony mortality, and from that day through the duration of the experiment there was no significant difference (P>0.05) in colony mortality among the four treatments.

A comparison of the LT_{50}'s and LT_{90}'s among treatments is shown in Table 19. The LT_{50}'s of all treatments were less than 3 d, with the exception of treatment consisting of bait placement 3 m radius from the mound, which had an LT_{50} of 3.4 d. The LT_{90}'s of all four treatments were very similar, as they were separated by only one half of a day, with the LT_{90}'s ranging from 5.0-5.5 d.

Table 19. Comparison of the LT_{50}'s and the LT_{90}'s for RIFA colonies that were treated by placing 10 g of 0.045% Advion™ around each mound in a circle with a radius of 0.5 m, 1 m, 2 m, or 3 m.

	LT_{50} and LT_{90} per treatment (d)			
Lethal time	Treatment 1- Advion™ 0.5 m from mound	Treatment 2- Advion™ 1 m from mound	Treatment 3- Advion™ 2 m from mound	Treatment 4- Advion™ 3 m from mound
LT_{50}	2.9	2.7	2.9	3.4
LT_{90}	5.3	5.0	5.2	5.5

Discussion and Conclusions

Though broadcast treatments with a proven bait are widely considered to be more efficient than individual mound treatments in terms of the cost/labor to benefit ratio, individual mound treatments are necessary and more practical in certain situations, such as when only a small area requires treatment or when special care must be taken to

ensure that non-target species are less likely to be effected by the treatment (Barr 1999; Barr and Best 1999; Drees et al. 1996). However, no research has been conducted and published on individual mound treatments with Advion™. Therefore, this research was conducted and data collected in an attempt to establish three important criteria with relation to achieving maximum control of the RIFA via the administration of individual mound treatments with Advion™: the most effective concentration and quantity of Advion™, as well as the most effective placement of the bait. For the two experiments that were conducted in order to establish the three previously mentioned criteria, as with any experimentation conducted on RIFA in the field it was important to ensure that the bait was placed out when the soil temperature was within the optimal foraging range of the ants (22-36° C) and when a rainfall event was not expected for one or more days following treatment (Drees et al. 1996; Vinson 1997). As shown in Tables 14 and 17, the mean soil temperature was always greater than 26.0° C, and there was no rainfall event within at least 2 d of treatment. These data indicated that the bait was available during peak foraging periods, and that the bait was not adversely affected by a rainfall event, thus validating the timing of treatment for both experiments.

In terms of the most effective concentration and quantity of Advion™ for use with individual RIFA mound treatments, this research revealed that several different concentrations and quantities of Advion™ were effective in achieving RIFA colony mortality of 90% or greater, though there were differences in the amount of time that was necessary for these various concentrations and quantities to achieve that level of colony mortality. As the data in Table 15 illustrated, after 14 d there was no significant

difference (P>0.05) in the percentage of colony mortality resulting from the following treatments: 5-20 g of 0.045% Advion™, 15-20 g of 0.016% Advion™, and 15-20 g of 0.008% Advion™. Furthermore, each of these treatments resulted in ~90% colony mortality or greater by day 14 of the experiment. Therefore, eight of the 12 concentrations and quantities of Advion™ that were evaluated in this experiment resulted in very effective control of the RIFA.

Though numerous concentrations and quantities of Advion™ proved to be effective at controlling the RIFA, there were significant differences (P<0.05) in the amount of time it took the various treatments to achieve 90% colony mortality. As shown in Tables 15 and 16, treatments with 10 g, 15 g, and 20 g of 0.045% Advion™ achieved 90% colony mortality more quickly than any of the other treatments, with LT_{90}'s of slightly more than 5 d for each of these three treatments. Two other treatments, 20 g of 0.016% Advion™ and 20 g of 0.008% Advion™, had resulted in 90% colony mortality after ~1 wk, though the LT_{90}'s of these two treatments were both determined to be greater than 8 d. Each of the other effective treatments had LT_{90}'s of greater than 9 d. Therefore, these data indicate that treatment with 10 g, 15 g, and 20 g of 0.045% Advion™ resulted in 90% colony mortality more quickly than all other treatments in this experiment. This clearly indicated that, at least up to 0.045%, there was a positive correlation between concentration and speed of colony mortality.

Beginning with the third day and lasting the duration of the experiment, there was no significant difference (P>0.05) among the three previously mentioned treatments that resulted in 90% colony mortality more quickly than the other treatments. This indicated

that that there was an apparent threshold at ~10 g of 0.045% Advion™, and quantities in excess of that amount were essentially no more rapid or effective in terms of colony mortality. In light of this, it is important to remember that when attempting to control pest insects with chemicals, it is always important to use the least amount of chemical that will still ultimately provide the maximum amount of control in the most desirable period of time (Pedigo 2002). Therefore, out of the three treatments that resulted in 90% colony mortality more quickly than all other treatments in this experiment, 10 g of 0.045% Advion™ would likely be the most appropriate concentration and quantity of Advion™ for use with individual RIFA mound treatments.

With relation to the most effective placement of Advion™ for individual RIFA mound treatments, this experiment revealed that there was little difference in colony mortality achieved or speed of colony mortality achieved based upon the placement of the bait. As shown in Table 18, by day 4 of the experiment there was no significant difference ($P>0.05$) among three of the four treatments in terms of the percentage of colony mortality achieved, and by day 5 no significant difference ($P>0.05$) existed among any of the four treatments. Further, the data in Table 19 revealed that the LT_{90}'s were very similar for these treatments, as all fell within the very narrow range of 5.0-5.5 d. Therefore, based on the data produced from this experiment, it was determined that there was no difference in terms of colony mortality achieved or speed of colony mortality achieved based upon the placement of 10 g of 0.045% Advion™ around a RIFA mound in a circle with a radius of 0.5 m, 1 m, 2 m, or 3 m.

In conclusion, the results of this experiment indicated that 10 g of 0.045% Advion™ was the most appropriate concentration and quantity of the eight concentrations and quantities (5-20 g of 0.045% Advion™, 15-20 g of 0.016% Advion™, and 15-20 g of 0.008% Advion™) that proved to be effective as individual RIFA mound treatments. Furthermore, the data indicated that there was no significant difference ($P>0.05$) in colony mortality when 10 g of 0.045% Advion™ was placed around a RIFA mound in a circle with a radius of 0.5 m, 1 m, 2 m, or 3 m. Resultantly, based upon the data produced from this research, it was determined that 10 g (2 Tbsp) of 0.045% Advion™ placed around a RIFA mound in a circle with a radius of 0.5-3.0 m was the most effective manner in which to treat an individual RIFA mound with Advion™.

Future research should include multiple concentrations of Advion™ other than those tested in this research, ideally those between 0.016% and 0.045%. It would also be ideal to take daily readings for the duration of the experiment, unlike in this research in which daily readings were not taken during the second week, to produce more precise data on colony mortality for those treatments that resulted in significant colony mortality during the second week. Additionally, the length of future experiments should be greater than the 14 d duration of the experiments in this study in order to determine longevity of RIFA control in areas subjected to individual mound treatments. It would then be possible to compare the data derived from such individual mound treatment experiments to data derived from experiments conducted on broadcast treatments with Advion™ in order to evaluate the differences in speed of colony mortality and longevity of control resulting from these two different treatment types.

CHAPTER IV

COMPARISON OF THE EFFECTIVENESS OF A LABEL-RATE BROADCAST TREATMENT WITH ADVION™, A LABEL-RATE BROADCAST TREATMENT WITH AMDRO®, AND A PRE-BAITING BROADCAST TREATMENT WITH ADVION™ AT CONTROLLING *Solenopsis invicta* BUREN

Introduction

Since its accidental introduction into the United States in the 1930's, a wide variety of chemicals and methods of delivering those chemicals have been utilized in an attempt to control the red imported fire ant (RIFA), *Solenopsis invicta* Buren (Banks 1990; Collins et al. 1992; Eden and Arant 1949; Lofgren et al. 1964; Lofgren et al. 1975; Phillips and Thorvilson 1989; Sauer et al. 1982; Vander Meer et al. 1982). Beginning in the 1960's and 1970's, the effectiveness of baits as a method of chemical delivery was realized, and their use became increasingly popular. Baits were very effective because they took advantage of the complex, trophallaxis-driven, social feeding behavior of the RIFA. These popular baits were composed of corncob grit-based granules, which were coated with some type of oil (most commonly soybean oil) to which the insecticide had been added (Banks 1990; Lofgren et al. 1964). Essentially, this same recipe for baits is still used today, and only the active ingredient has changed.

Over the years, a number of different chemicals have been delivered via baits in an attempt to control the RIFA. These chemicals range from the GABA-gated chloride

channel antagonist, mirex, which was used in the 1960's and 1970's, to chemicals that are being used at the present time, such as the type II electron transport inhibitor, hydramethylnon, and the juvenile hormone mimic, fenoxycarb (Collins et al. 1992; Lofgren et al. 1975; Phillips and Thorvilson 1989; Vander Meer et al. 1982). However, due to RIFA resiliency, as well as the United States Environmental Protection Agency's increasingly strict regulations, a continuous need to develop new and effective chemical control measures exists. The newest chemical available for the control of the RIFA is indoxacarb. Classified as an oxadiazine, which is a new class of pyrazoline-type insecticides, the chemical indoxacarb was discovered by E.I. DuPont de Nemours and Company in 1991 (McCann et al. 2001). Indoxacarb is a novel insecticide that must first be metabolized in order to become acutely toxic. Following ingestion by the insect, metabolic breakdown of indoxacarb occurs, ultimately producing an N-decarbomethoxyllated metabolite known as JT333. It is JT333 that is so highly toxic to the insect, functioning as a potent, voltage-dependent sodium channel blocker that ultimately results in the relatively rapid death of the insect (Wing et al. 2000).

Registered by the United States Environmental Protection Agency in August of 2004 (EPA Registration #352-627), one of the newest baits available for RIFA control is Advion™ (E.I. DuPont de Nemours and Company, Wilmington, DE), which contains the aforementioned active ingredient indoxacarb. As with many RIFA baits, Advion™ can either be applied as a broadcast treatment or as an individual mound treatment. While both methods have the potential to effectively achieve control of the RIFA, individual mound treatments are generally more expensive and more labor intensive than

broadcast treatments due to the fact that a greater amount of bait is usually necessary to attain equivalent control, and the fact that each mound in an area must be treated individually. Though there are some circumstances in which individual mound treatments are more practical and appropriate, broadcast treatments are widely considered to be more efficient than individual mound treatments in terms of the cost/labor to benefit ratio (Barr 1999; Barr and Best 1999; Barr et al. 1999; Drees et al. 1996). Thus RIFA baits are most commonly administered as broadcast treatments. Therefore, this research was focused on evaluating the effectiveness of broadcast treatment with the new RIFA bait Advion™, as well as comparing the effectiveness of such treatment to other broadcast treatments aimed at controlling the RIFA.

Specifically, the purpose of this research was to compare the effectiveness of label-rate broadcast treatment with Advion™ to the effectiveness of two other broadcast treatments: label-rate broadcast treatment with the popular hydramethylnon-containing bait, Amdro®, and pre-baiting broadcast treatment involving both a placebo and Advion™. To date, only limited research comparing the effectiveness of broadcast treatment with Advion™ to the effectiveness of broadcast treatment with other baits has been conducted and published (Barr 2003). Furthermore, no research has been conducted and published on the effectiveness of Advion™, or any other RIFA bait, when used in a pre-baiting broadcast treatment. Pre-baiting, which involves treatment with non-toxic bait in order to stimulate feeding activity prior to treatment with toxic bait, has already proven to be effective at controlling numerous pest species (Shumake et al. 2002; Sterner 1999). Therefore, this research was conducted to compare the

effectiveness of label-rate broadcast treatment with Advion™ to the effectiveness of both label-rate broadcast treatment with the RIFA bait Amdro® (Ambrands, Atlanta, GA), and pre-baiting broadcast treatment with Advion™.

Materials and Methods

This experiment was conducted in the field on a private game ranch (N28°05’79” W98°05’75”) located in Jim Wells County, Texas. The property was ~4856 ha, with the majority of the land groomed for dove, quail and deer hunting. A considerable portion of the land was utilized for cattle grazing, as well. Though there were a variety of different types of vegetation throughout the property, this experiment was conducted on a 5 ha field consisting of grass pastureland. No cattle grazing was allowed on this field immediately preceding the experiment, during the experiment, or immediately following the experiment. The grass comprising this 5 ha field was mowed ~1 wk prior to the beginning of the experiment and was then allowed to grow unimpeded for the duration of the experiment.

RIFA mounds were selected for use in this experiment by first placing an individually numbered, 50 cm tall, metal wire, fluorescent colored flag in the center of a given mound. Once placed in the mound, the metal wire was vibrated to elicit a response from the RIFA and determine the activity level of the colony. The following Lichert scale was used to determine the activity level: “0” = inactive (no ants responding), “1” = minor activity (1-50 ants responding), “2” = moderate activity (51-100 ants responding) and “3” = fully active (more than 100 ants responding). Only colonies with a Lichert

scale rating of “3” were selected for use in this experiment (Gold et al. 1996a, 1996b). All RIFA mounds on the 5 ha field that was used for this experiment were measured for colony activity in this manner.

Next, 15 rectangular-shaped 0.13 ha plots were measured via the use of a Rolatape® M300 series measuring wheel (Rolatape Corp., Spokane, WA). Each plot was separated from the next nearest plot by at least 15 m. A 46 cm x 5 cm x 2 cm wooden stake was then hammered into the soil at each of the four corners of a given plot, and #16 polyester/cotton twine was used to connect one stake to the next, thus forming a clearly demarcated rectangular border. At least 10 RIFA mounds with an initial Lichert scale rating of “3” were located within the borders of each of these 15 plots.

For this experiment, there were four different treatment groups and a control group. Treatment 1 consisted of broadcast treatment with 0.0% Advion™ (contained no indoxacarb), Treatment 2 consisted of broadcast treatment with 0.73% Amdro®, Treatment 3 consisted of pre-baiting broadcast treatment comprised of broadcast treatment with 0.0% Advion™ (contained no indoxacarb) followed one hour later by broadcast treatment with 0.045% Advion™, Treatment 4 consisted of broadcast treatment with 0.045% Advion™, and the control group consisted of untreated colonies. Treatment 1, which consisted of broadcast treatment with 0.0% Advion™, was included in this experiment as a treatment group instead of a control group due to the fact that there has previously been no research conducted to analyze any potential insecticidal qualities that the inert ingredients within Advion™ might exhibit. Therefore, this treatment group was included to ensure that the mortality resulting from treatment with

Advion™ resulted solely from the active ingredient indoxacarb. There were three replicates for each of the four treatment groups and the control group, thus a total of 15 plots was utilized for this experiment. As previously mentioned, each plot contained at least 10 mounds with an initial Lichert scale rating of "3".

Experimentation was conducted for a total of 7 wk (June 4-July 23, 2004). Between approximately 7:00 a.m. and 9:00 a.m. C.S.T. on the first morning of the experiment, broadcast treatments were administered at label rates (1.7 kg/ha) via the use of Scotts® Handy Green II® hand spreaders (Scotts Company, Marysville, OH). Separate hand spreaders were used for each of the different treatments. For Treatment 1, 227 g of 0.0% Advion™ was broadcast over each plot, and 227 g of 0.73% of Amdro® was broadcast over each plot for Treatment 2. For Treatment 3, 227 g of 0.0% Advion™ was broadcast over each plot, and then ~1 h later 227 g of 0.045% Advion™ was broadcast over each of those same plots. For Treatment 4, 227 g of 0.045% Advion™ was broadcast over each plot and, as previously mentioned, the plots for the control group were left untreated. The bait used for each treatment was carefully weighed using an Ainsworth® 6000 g electric scale, model APX-6001 (Denver Instrument Company, Denver, CO), and placed into separate, sealed, plastic bags the morning of treatment to ensure that each plot within a given treatment group received an identical amount of bait.

The next morning, ~24 h following treatment, the metal wire of the flag that had been placed in each mound was vibrated to elicit a response from the RIFA and measure the activity level of the colony. The response by the ants was graded according to the Lichert scale previously described, and colony mortality was assumed when a response

of “0” was observed. Data was gathered in this manner each day for the first 7 d, with an additional reading for each colony being taken 2 wk later, and one final reading for each colony being taken 4 wk from the previous reading. Thus, ultimately, a data set of colony mortality was produced for each treatment. Longevity of control afforded by each treatment was also measured via daily observations of any new RIFA mounds appearing within the plots.

Additionally, the soil temperature and precipitation were measured during this experiment. Soil temperature was measured with a Sergeant-Welch 12.7 cm soil thermometer (Sergeant-Welch, Buffalo Grove, IL) each morning within each of the plots for the first 7 d of the experiment, and then again on the two subsequent mornings when additional colony activity readings were taken. Precipitation was measured with a Garden Treasures® 15.2 cm capacity rain gauge (Lowe’s Companies Inc., Wilkesboro, NC) for each 24 h period for the first 7 d of the experiment. No 24 h precipitation readings were taken after the first 7 d.

Statistics. At the conclusion of this experiment, SPSS® software (SPSS 2001) was used to conduct statistical analysis of the data. First, however, Abbott’s formula was used to correct all mortality data (Abbott 1925). Abbott’s formula for correcting mortality data is

$$[(X-Y) / X] \times 100 = \text{percent control}$$

where “X” is the percent colony survival in the control group, and “Y” is the percent

colony survival in the treatment group. Next, ANOVA was conducted on the corrected mortality data sets. Finally, the LSD post hoc test was conducted on those same data sets to determine significant differences among treatments. All tests of significance were evaluated at $P = 0.05$.

Results

The mean morning soil temperature and the daily precipitation are shown in Table 20. The mean morning soil temperature was always 26.0° C or greater, and no precipitation occurred within 2 d of when the broadcast treatments were conducted. A comparison among treatments of the mean cumulative daily colony mortality is shown in Table 21 and Figure 11 ($F=4.28$; $df=35$; $P<0.05$). For days 2-7 of the experiment, significantly greater ($P<0.05$) RIFA colony mortality resulted from broadcast treatment with 0.045% Advion™ than from any of the other three treatments. Pre-baiting broadcast treatment resulted in significantly greater ($P<0.05$) colony mortality during that same time period than all other treatments except for the broadcast treatment with 0.045% Advion™. No colony mortality resulted from broadcast treatment with 0.73% Amdro® until day 5 of the experiment, and no colony mortality resulted from broadcast treatment with 0.0% Advion™ during the entire first week. By day 21, both broadcast treatment with 0.045% Advion™ and pre-baiting broadcast treatment had essentially eliminated all colonies within their respective plots, and there was no significant difference ($P>0.05$) in mortality between the two treatments. Additionally, both of the previously mentioned

Table 20. Mean morning soil temperature and daily precipitation for the experiment that consisted of label-rate broadcast treatment with 0.045% Advion™, 0.73% Amdro®, and 0.0% Advion™ (contained no indoxacarb), and pre-baiting broadcast treatment consisting of treatment with 0.0% Advion™ (contained no indoxacarb) followed 1 h later by treatment with 0.045% Advion™.

Day	Mean morning soil temperature ± SD (ºC)	Daily precipitation (cm)
0	28.4±0.7	0.0
1	27.7±0.6	0.0
2	27.1±0.9	0.0
3	26.6±0.7	<0.3
4	26.2±0.9	3.0
5	26.0±0.6	0.3
6	26.3±0.7	1.3
7	26.5±0.4	<0.3
21	26.6±0.4	—[a]
49	27.4±0.7	—[a]

[a] A precipitation reading was not taken on this day.

Table 21. Comparison of the mean percent RIFA colony mortality resulting from label-rate broadcast treatment with 0.045% Advion™, 0.73% Amdro®, and 0.0% Advion™ (contained no indoxacarb), and from pre-baiting broadcast treatment consisting of treatment with 0.0% Advion™ (contained no indoxacarb) followed 1 h later by treatment with 0.045% Advion™.

Day	Mean cumulative percent colony mortality per treatment[a] (mean ± SD)			
	Treatment 1- plots treated with 227 g of 0.0% Advion™	Treatment 2- plots treated with 227 g of 0.73% Amdro®	Treatment 3- plots treated first with 227 g of 0.0% Advion™, then with 227 g of 0.045% Advion™	Treatment 4- plots treated with 227 g of 0.045% Advion™
1	0.0±0.0a	0.0±0.0a	0.0±0.0a	6.1±5.3a
2	0.0±0.0a	0.0±0.0a	13.8±8.1b	45.5±9.1c
3	0.0±0.0a	0.0±0.0a	28.4±2.0b	63.6±9.1c
4	0.0±0.0a	0.0±0.0a	39.6±5.7b	75.8±10.5c
5	0.0±0.0a	6.4±5.5a	56.4±12.0b	84.9±10.5c
6	0.0±0.0a	28.3±5.2b	60.9±8.5c	87.1±6.6d
7	0.0±0.0a	43.4±7.1b	69.5±7.9c	96.2±6.6d
21	6.1±5.3a	85.9±10.6b	98.4±1.4c	99.2±1.4c
49	6.5±5.7a	86.7±9.9b	98.4±1.4c	99.2±1.4c

[a] Mortality was corrected by the formula in Abbott (1925). Means within a row with different letters are significantly different at $P<0.05$. Means were separated using the LSD test.

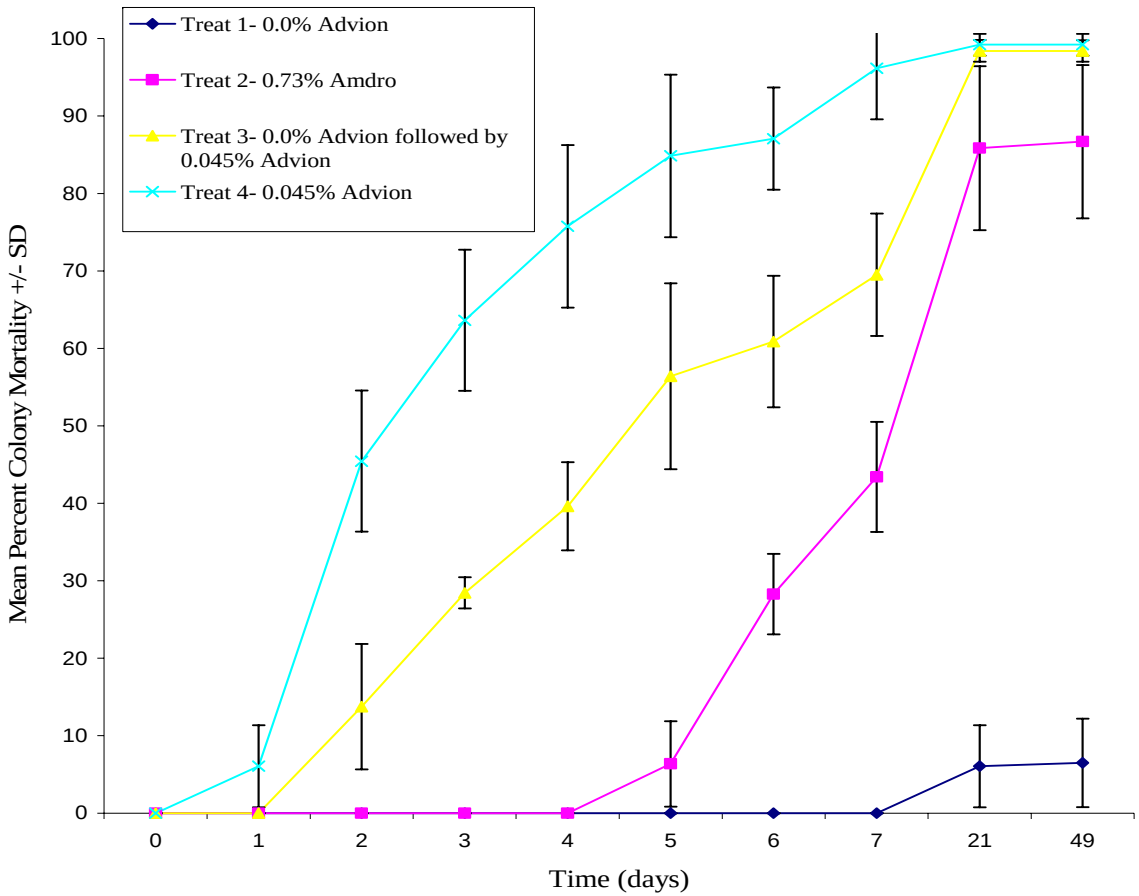

Figure 11. A comparison of the mean percent RIFA colony mortality resulting from label-rate broadcast treatment with 0.045% Advion™, 0.73% Amdro®, and 0.0% Advion™ (contained no indoxacarb), and from pre-baiting broadcast treatment consisting of treatment with 0.0% Advion™ (contained no indoxacarb) followed 1 h later by treatment with 0.045% Advion™.

treatments had resulted in significantly greater ($P<0.05$) colony mortality by day 21 than broadcast treatment with 0.73% Amdro®, and there was no change in these data by day 49.

A comparison of the LT_{50}'s and LT_{90}'s among treatments is shown in Table 22. The LT_{50} resulting from broadcast treatment with 0.045% Advion™ (LT_{50}=2.4 d) was one half the corresponding LT_{50} resulting from pre-baiting broadcast treatment (LT_{50}=4.7 d), and both of those LT_{50}'s were considerably less than that resulting from broadcast treatment with 0.73% Amdro® (LT_{50}=9.7 d). Similarly, the LT_{90} resulting from broadcast treatment with 0.045% Advion™ (LT_{90}=6.2 d) was one half the corresponding LT_{90} resulting from pre-baiting broadcast treatment (LT_{90}=11.7 days). Broadcast treatment with 0.73% Amdro® did not attain an LT_{90}, and broadcast treatment with 0.0% Advion™ did not attain an LT_{50} or an LT_{90}.

A comparison of the mean number of new RIFA mounds appearing in the plots of the four treatment groups and the control group at days 21 and 49 is shown in Table 23 ($F=7.11$; $df=9$; $P<0.05$). After 21 d, there was little difference among the groups, as four out of the five groups exhibited no significant difference ($P>0.05$) in terms of the number of new RIFA mounds per plot. Additionally, all four of those groups had a mean of less than one new RIFA mound per plot after 21 d, and the plots that were subjected to either broadcast treatment with 0.045% Advion™ or pre-baiting broadcast treatment contained no new RIFA mounds. However, after 49 d, the control group and the treatment group consisting of broadcast treatment with 0.0% Advion™ had a significantly greater ($P<0.05$) number of new RIFA mounds per plot than the other

Table 22. Comparison of the LT_{50}'s and the LT_{90}'s for RIFA colonies that were treated via label-rate broadcast treatment with 0.045% Advion™, 0.73% Amdro®, and 0.0% Advion™ (contained no indoxacarb), and via pre-baiting broadcast treatment consisting of treatment with 0.0% Advion™ (contained no indoxacarb) followed 1 h later by treatment with 0.045% Advion™.

Lethal time	LT_{50} and LT_{90} per treatment (d)			
	Treatment 1- plots treated with 227 g of 0.0% Advion™	Treatment 2- plots treated with 227 g of 0.73% Amdro®	Treatment 3- plots treated first with 227 g of 0.0% Advion™, then with 227 g of 0.045% Advion™	Treatment 4- plots treated with 227 g of 0.045% Advion™
LT_{50}	—[a]	9.7	4.7	2.4
LT_{90}	—[b]	—[b]	11.7	6.2

[a] An LT_{50} was not attained during this experiment.
[b] An LT_{90} was not attained during this experiment.

Table 23. The mean cumulative number of new RIFA mounds that were observed per plot following label-rate broadcast treatment with 0.045% Advion™, 0.73% Amdro®, and 0.0% Advion™ (contained no indoxacarb), and pre-baiting broadcast treatment consisting of treatment with Advion™ (contained no indoxacarb) followed 1 h later by treatment with 0.045% Advion™.

Day	Mean cumulative number of new RIFA mounds per plot [a b] (Mean ±SD)				
	Treatment 1- plots treated with 227 g of 0.0% Advion™	Treatment 2- plots treated with 227 g of 0.73% Amdro®	Treatment 3- plots treated first with 227 g of 0.0% Advion™, then with 227 g of 0.045% Advion™	Treatment 4- plots treated with 227 g of 0.045% Advion™	Control- plots left untreated
21	1.3±0.6a	0.3±0.6b	0.0±0.0b	0.0±0.0b	0.7±0.6ab
49	2.7±0.6a	1.3±0.6b	0.0±0.0c	0.3±0.6c	3.0±0.0a

[a] No new RIFA mounds were observed on any of the plots for days 1-7 of the experiment. Therefore, only new RIFA mounds observed on days 21 and 49 were used in this analysis.
[b] Means within a row with different letters are significantly different at $P<0.05$. Means were separated using the LSD test.

three groups, with each having three new RIFA mounds per plot. Though the treatment group consisting of broadcast treatment with 0.73% Amdro® had significantly fewer ($P<0.05$) new RIFA mounds per plot than both the control group and the treatment group consisting of broadcast treatment with 0.0% Advion™, it had a significantly greater ($P<0.05$) number of new RIFA mounds per plot than the treatment groups consisting of broadcast treatment with 0.045% Advion™ and pre-baiting broadcast treatment. There was no significant difference in the number of new RIFA mounds per plot in these latter two treatments and, at 0.3 and 0.0 new mounds per plot respectively, they had significantly fewer ($P<0.05$) new RIFA mounds per plot than the other two treatment groups and the control group.

Discussion and Conclusions

As with any new RIFA bait, it is necessary to evaluate the effectiveness of broadcast treatment with Advion™, and to compare it to broadcast treatment with other RIFA baits. Furthermore, it is practical to investigate other potentially more effective methods of conducting broadcast treatments, such as the utilization of pre-baiting broadcast treatments, which have proven to be effective against numerous pest species (Shumake et al. 2002, Sterner 1999). For these reasons, this research was conducted, thus producing data on the effectiveness of label-rate broadcast treatment with Advion™, its effectiveness in comparison to label-rate broadcast treatment with another RIFA bait (Amdro®), and its effectiveness in comparison to pre-baiting broadcast treatment with Advion™.

For this experiment, as with all experimentation conducted on RIFA in the field, it was important to ensure that all bait was broadcast when the soil temperature was within the optimal foraging range of the ants (22-36° C) and when a rainfall event was not expected for one or more days following treatment (Drees et al. 1996; Vinson 1997). As shown in Table 20, the mean soil temperature was always between 26° C and 29° C, and there was no rainfall event within 2 d of when the broadcast treatments were conducted. These data indicated that the bait was available during peak foraging periods, and that the bait was not adversely affected by a rainfall event, thus validating the timing of treatment for this experiment.

As was clearly shown by the data in Tables 21 and 22, broadcast treatment with 0.045% Advion™ resulted in 90% colony mortality far more quickly than any of the other treatments. The next nearest treatment in terms of overall colony mortality and speed of mortality was pre-baiting broadcast treatment, which took nearly twice as long to achieve 90% colony mortality as broadcast treatment with 0.045% Advion™. However, though it took longer to achieve, pre-baiting broadcast treatment ultimately resulted in the same overall level of RIFA colony control as the aforementioned broadcast treatment with 0.045% Advion™, as there was no significant difference ($P>0.05$) between the two treatments by the third week of the experiment. Essentially, though the total number of granules that is ultimately spread out over a given land area via label-rate broadcast treatment is relatively small even when making two applications of bait, as was done with the pre-baiting broadcast treatment, there appears to be a dilution effect with such a treatment. This was likely the reason that, even though pre-

baiting broadcast treatment ultimately achieved the same level of RIFA colony mortality as the highly effective label-rate broadcast treatment with 0.045% Advion™, it took nearly twice as long to attain that level.

Broadcast treatment with 0.73% Amdro®, which ultimately achieved ~87% colony mortality over the duration of this experiment, resulted in significantly less ($P<0.05$) colony mortality than either of the two aforementioned treatments. Further, the LT_{50} resulting from treatment with Amdro® was more than twice that of the pre-baiting broadcast treatment and more than four times that of label-rate broadcast treatment with Advion™. Broadcast treatment with 0.0% Advion™ resulted in no colony mortality for the first 7 d and, ultimately, RIFA colony mortality only reached ~6%, which was not significantly different than the untreated control group. The data from this broadcast treatment with 0.0% Advion™ indicated, therefore, that the insecticidal qualities of Advion™ did in fact result solely from the chemical indoxacarb. Resultantly, based on this information and all of the data derived from the four different treatment groups, label-rate broadcast treatment with 0.045% Advion™ was the most effective overall in terms of RIFA colony mortality and speed of mortality.

The data in Table 23 indicated that there was little difference between treatments in terms of the number of new RIFA mounds per plot observed 21 d after treatment. However, there were significant differences in the number of new RIFA mounds per plot 49 d after the broadcast treatments were conducted. Both the control group and the treatment group consisting of broadcast treatment with 0.0% Advion™ had a mean of three new RIFA mounds per plot by day 49, and this was significantly greater ($P<0.05$)

than the number of new RIFA mounds per plot in any of the other three groups. Though there were only 1.3 new RIFA mounds per plot by day 49 in the treatment group consisting of broadcast treatment with Amdro®, that was still significantly greater ($P<0.05$) than the number of new mounds per plot that were observed in the treatment groups consisting of broadcast treatment with 0.045% Advion™ and pre-baiting broadcast treatment. After 49 d, only one new RIFA mound was observed on all of the plots subjected to the latter two treatments and, resultantly, those two treatment groups had significantly fewer ($P<0.05$) new RIFA mounds per plot than any of the other treatment groups.

In conclusion, both pre-baiting broadcast treatment and label-rate broadcast treatment with 0.045% Advion™ ultimately resulted in the same high level of RIFA colony mortality (98-99%), which was significantly greater ($P<0.05$) than that resulting from the other treatments. Further, there was no significant difference ($P>0.05$) in the mean number of new RIFA mounds observed per plot over the course of this experiment for the two aforementioned treatment groups. However, though pre-baiting broadcast treatment and broadcast treatment with 0.045% Advion™ ultimately achieved the same level and duration of RIFA control, broadcast treatment with 0.045% Advion™ was determined to be the most effective treatment overall due to the fact that it resulted in an LT_{90} of only 6.2 d, which was approximately half the LT_{90} resulting from pre-baiting broadcast treatment.

In order to further validate these results, this experiment should be repeated. In future research, it would be ideal to use various quantities of 0.0% Advion™ in the pre-baiting

experiment to determine whether pre-baiting is, in fact, less effective due to a dilution effect. Additionally, the length of time it takes for the RIFA to re-colonize an area that has been subjected to broadcast treatment with a bait is highly variable, and largely dependent upon the number of RIFA colonies located on the land adjacent or in close proximity to the treatment area (Drees et al. 1996). Although for this experiment there was only one new RIFA colony observed after 49 d on the plots that were treated in some manner (i.e. pre-baiting broadcast treatment or label-rate broadcast treatment) with 0.045% Advion™, numerous studies of varied duration in various environments need to be conducted to definitively determine the longevity of control afforded by label-rate broadcast treatment with 0.045% Advion™.

CHAPTER V

TROPHALLACTIC TRANSMISSION AND METABOLISM OF THE ACTIVE INGREDIENT INDOXACARB IN ADVION™

Introduction

The red imported fire ant (RIFA), *Solenopsis invicta* Buren, is a eusocial insect with a caste system consisting of brood, workers, and reproductives. The brood caste consists of eggs, larvae, and pupae. The entirely female worker caste consists primarily of foragers and nurses, and the reproductive caste consists of both males and females which are capable of reproduction. Each of these castes is involved in the complex process of social feeding, which begins with the distribution of food throughout the entire colony via trophallaxis. Though all types of food undergo distribution throughout the colony, the sequence of this distribution is different when comparing liquid to solid foods. The general pathway involving liquid foods, such as oils, begins with the foragers first feeding the nurses and other workers via trophallaxis. The nurses then feed the larvae and queen via trophallaxis. This pathway is relatively simple, as all caste members are capable of digesting liquid foods (Cassill and Tschinkel 1995; Lofgren et al. 1975; Vinson 1983; Vinson 1997).

The general pathway involving solid foods, such as protein, is more complex, as the only caste members that can digest solid foods are the fourth instar larvae. Therefore, the foragers begin by passing the solid food to the nurses, who then pass the solid food

along to the fourth instar larvae. These larvae digest the solid food and, via trophallaxis, pass now-liquified food back to the nurses. By way of trophallaxis, the nurses then feed this now-liquefied food to the other larval instars, the queen, and the other workers. It is through these two unique, trophallaxis-driven pathways that the entire colony is ultimately supplied with all necessary nutrients (Cassill and Tschinkel 1995; Lofgren et al. 1975; Vinson 1983; Vinson 1997).

Though a variety of chemicals and methods of delivering these chemicals have been used in an attempt to control the RIFA since its introduction into the United States in the 1930's, baits have been an extremely popular and effective delivery method since they were first used for RIFA control in the 1960's (Banks 1990; Collins et al. 1992; Lofgren et al. 1964; Lofgren et al. 1975; Phillips and Thorvilson 1989; Vander Meer et al. 1982). Baits are very effective because they take advantage of the previously described complex, trophallaxis-driven, social feeding behavior of the RIFA. These popular baits consist of both a liquid and a solid component, being commonly composed of corncob grit-based granules, which are coated with some type of oil (most commonly soybean oil) to which the insecticide has been added (Banks 1990; Lofgren et al. 1964). Baits are used either as individual mound treatments or, more commonly, as broadcast treatments. Regardless of which of these two types of bait treatments is used, once the bait has been placed out in the field, RIFA foragers locate it and carry it back to the colony where feeding commences. The bait contents, essentially the oil and the insecticide, are then passed to the various caste members via trophallaxis, ultimately destroying the colony (Drees et al. 1996).

Registered by the United States Environmental Protection Agency in August of 2004 (EPA Registration #352-627), Advion™ (E.I. DuPont de Nemours and Company, Wilmington, DE) is one of the newest baits available for the control of the RIFA. Advion™ contains the active ingredient indoxacarb, which is classified as an oxadiazine, a new class of pyrazoline-type insecticides (McCann et al. 2001). Discovered by E.I. DuPont de Nemours and Company in 1991, the chemical indoxacarb is a novel insecticide that must first be metabolized in order to become acutely toxic. Following ingestion by the insect, metabolic breakdown of indoxacarb occurs via amidase and esterase enzymes commonly found within the midgut and/or fat bodies, ultimately producing an N-decarbomethoxyllated metabolite known as JT333. It is JT333 that is so highly toxic to the insect, functioning as a potent, voltage-dependent sodium channel blocker. The bio-activation of this potent metabolite within the insect ultimately results in severe neurotoxic symptoms, paralysis, and a relatively rapid death (Wing et al. 2000).

In order for a bait to be effective, it is essential for the insecticide within the bait to be rapidly passed throughout the colony via trophallaxis (Collins & Callcott 1998). Though it is assumed that the indoxacarb within Advion™ is passed among the various RIFA caste members via trophallaxis, no formal research has been conducted that visually demonstrates the occurrence of this process. Furthermore, it is believed that indoxacarb is metabolized into JT333 by the RIFA larvae, with the metabolite then being passed to the other caste members which ultimately results in the death of the colony (E.I. DuPont de Nemours and Company 2004). No research has been conducted to determine whether

the members of the worker caste are actually capable of metabolizing indoxacarb into the toxic metabolite themselves. The purpose of this research was to demonstrate that the indoxacarb within Advion™ is passed via trophallaxis, and to determine whether the RIFA worker caste is capable of metabolizing indoxacarb into the toxic JT333.

Materials and Methods

The following experiment was conducted in the laboratory, and all of the RIFA colonies used in this experiment were collected from the USDA-ARS Pecan Breeding Orchard (N30°37'21" W96°21'34") located in Brazos County, Texas. This location was chosen as the collection site due to the fact that no pesticides have been used on the land. Prior to the experiment, several RIFA colonies were excavated and placed into 12 liter plastic buckets that had been lined with talcum powder to prevent the ants from escaping. The colonies were transported back to the laboratory, dripped out, and then placed into 40 cm x 27 cm x 9.5 cm plastic sweater boxes (First Phillips Manufacturing, Leominster, MA) that were lined with Fluon® (Northern Products, Inc., Woonsocket, RI) to prevent the ants from escaping (Sorensen and Vinson 1981; Weeks et al. 2004).

Each sweater box contained a 14 cm x 2.5 cm petri dish, a 7.5 cm x 2 cm plastic weigh dish, and two 4 cm x 0.8 cm plastic weigh dishes. The petri dish served as the artificial brood chamber for the ants. It was filled with 1.5 cm of Castone® Dental Stone (Dentsply International, York, PA), which had been moistened with water prior to placing the ants into the sweater box, and the plastic lid on top of the petri dish contained two 3 cm holes which been cut into the lid to allow the RIFA easy access to the brood

chamber. The large weigh dish inside the sweater box contained cotton saturated with water, while one small weigh dish contained cotton saturated with a 20% sugar water solution, and the other small weigh dish contained live tenebrionid beetle larvae (Banks et al. 1981; Cassill and Tschinkel 1999; Sorensen and Vinson 1981).

Immediately after the ants had been placed into their respective sweater boxes, each colony was tested to determine if the colony contained the microsporidial protozoan, *Thelohania solenopsae*. The presence of these protozoans could indicate an unhealthy colony, and the use of such a colony in a laboratory experiment would likely provide unreliable data. In order to test for the presence of *T. solenopsae*, 30 workers were taken from a given colony, placed in a 0.5 ml Eppendorf® microcentrifuge tube (Eppendorf North America, Westbury, NY) to which 50 µl of deionized water had been added via a Rainin® EDP2™ 25-250 µl electronic pipette (Rainin Instrument, LLC, Oakland, CA), and macerated with an Eppendorf® micropestle (Eppendorf North America, Westbury, NY). Then, 30 µl of the newly formed homogenate was placed on a 2.5 cm x 7.5 cm x 1.0 mm glass slide and allowed to air-dry for 24 h (Keck 2005). After 24 h, the staining steps as outlined by Weber et al. (1992) were followed; and after another air-drying period of ~24 h, it was then possible to view the slide under a light microscope and determine whether or not *T. solenopsae* spores were present. The entire testing procedure took ~2 d.

After testing all colonies for the presence of *T. solenopsae*, a single *T. solenopsae*-free colony was selected for use, and this colony was maintained in a similar manner to that described by Banks et al. (1981). This included keeping the ants in an environment

of continuous light at a constant temperature of ~28° C, providing them with a constant supply of water, and maintaining them on a diet of 20% sugar water solution and tenebrionid beetle larvae. The RIFA were maintained in this manner for a total of 5 d to allow the ants a sufficient amount of time to become acclimated to the laboratory conditions (Collins and Callcott 1998). At the end of day 5, the two aforementioned small weigh dishes that contained food were removed from the sweater box that housed the colony. The RIFA were starved for 2 d to ensure that the ants' crops were emptied (Cassill and Tschinkel 1999).

On the second day without food, ants were removed from the common sweater box and separated into treatment and control groups. For this experiment, there were a total of four different groups: two treatment groups and two control groups. There were six replicates each for Treatment 1 and Treatment 2, and three replicates each for Control 1 and Control 2. Treatment 1 consisted of 1000 workers per replicate, Treatment 2 consisted of 1000 workers and ~250 brood per replicate, Control 1 consisted of 1000 workers per replicate, and Control 2 consisted of 1000 workers and ~250 brood per replicate.

In order to attain the 1000 RIFA workers for each replicate, individual workers were aspirated from the original colony housed in the sweater box with a BioQuip® model 1135A mouth aspirator (BioQuip Products, Inc., Rancho Dominguez, CA). An Ainsworth® 6000 g electric scale, model APX-6001 (Denver Instrument Company, Denver, CO), was used to weigh 0.125 g of brood (~250 brood) for those replicates which contained both workers and brood. After the aspirating and weighing had been

completed for a given replicate, the RIFA were placed into a 31 cm x 16.5 cm x 9.5 cm plastic shoe box (First Phillips Manufacturing, Leominster, MA) that was lined with Fluon®. Within each plastic shoe box was a 9 cm x 1.5 cm petri dish containing 0.75 cm of Castone® dental stone which served as the artificial brood chamber, and a 7.5 cm x 2 cm plastic weigh dish filled with water-soaked cotton which was constantly replenished for the duration of the experiment (Figure 12). A total of 18 shoe boxes were ultimately appropriated in this manner.

On the same day in which the previously mentioned groups of ants were separated into treatment and control groups, 18 sets of 100 workers each were aspirated from the original colony housed in the sweater box and spray painted using a technique similar to that described by Forschler (1994). After being aspirated from the original colony, a given set of 100 workers was placed into a 14 cm x 2.5 cm Fluon®-lined plastic petri dish, which was itself then placed in the bottom of a 61 cm x 51 cm x 31.5 cm cardboard box. The ants were then spray painted using Krylon® Fluorescent Pink Indoor/Outdoor Paint (Sherwin Williams Company, Cleveland, OH). Special care was taken to spray the paint at an angle near the top of the box to allow the paint to drift down into the petri dish, as opposed to direct spraying which could result in harmful saturation (Narayanan 2004). After being painted, each set of 100 ants was placed into a separate 9.5 cm x 6.5 cm Fluon®-lined plastic cylindrical container, which contained a 4 cm x 0.8 cm weigh dish filled with water-soaked cotton. In this manner, by the end of the second day that the original colony had been without food, there were 18 plastic shoeboxes containing

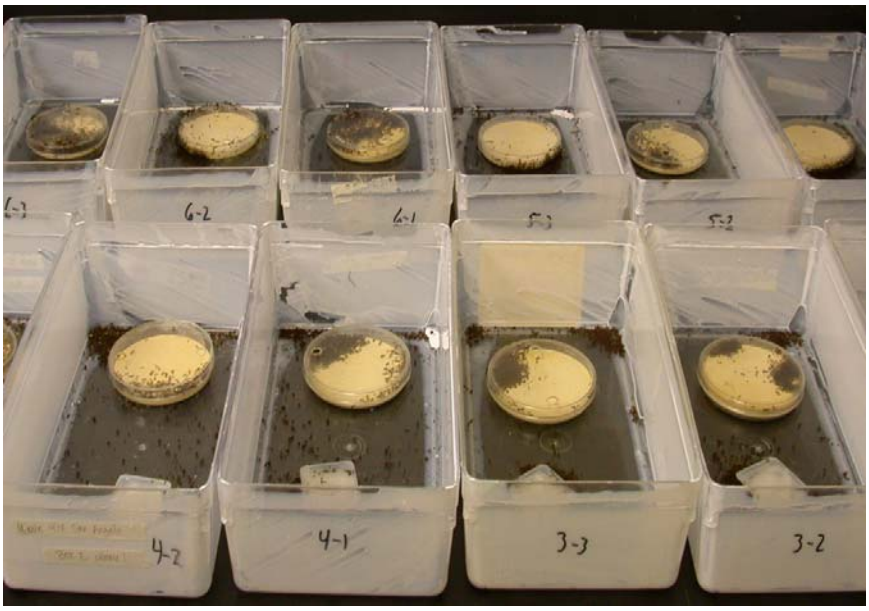

Figure 12. A group of Fluon®-lined 31 cm x 16.5 cm x 9.5 cm plastic shoe boxes used for the experiment on trophallactic transmission and metabolism of the active ingredient indoxacarb in Advion™. Within each plastic shoe box was a 9 cm x 1.5 cm petri dish containing 0.75 cm of Castone® dental stone which served as the artificial brood chamber, a 7.5 cm x 2 cm plastic weigh dish filled with water-soaked cotton which was constantly replenished for the duration of the experiment, and either 1000 RIFA workers or 1000 RIFA workers and ~250 brood.

either 1000 RIFA workers or 1000 RIFA workers and ~250 brood each, and 18 plastic cylindrical containers housing 100 spray painted RIFA workers each.

The next day the experiment was begun, and it lasted for a total of 30 d (May 9-June 8, 2005). In order to visually determine whether the indoxacarb within Advion™ was being passed among the caste members via trophallaxis, it was necessary to use dyed bait for this experiment. In previous research, Calco blue-dyed bait and Calco red-dyed bait were successfully utilized to study feeding activity of the RIFA (Bartlett and Lofgren 1961; Summerlin et al. 1975). Therefore, for this experiment, Calco blue-dyed

0.045% Advion™ and Calco blue-dyed 0.0% Advion™ (contained no indoxacarb) were utilized. Specifically, a single 4 cm x 0.8 cm weigh dish containing 1 g of Calco blue-dyed 0.045% Advion™ was placed into each of 12 of the plastic cylindrical containers that housed 100 spray painted RIFA workers each, and a single 4 cm x 0.8 cm weigh dish containing 1 g of Calco blue-dyed 0.0% Advion™ was placed into each of the six remaining plastic cylindrical containers (Figure 13). The painted RIFA workers within each plastic cylindrical container were then allowed to feed upon the bait for 5 h. All of the Calco blue-dyed bait used for this experiment was freshly produced and shipped directly from E.I. DuPont de Nemours and Company's research laboratory in Newark, Delaware.

After the 18 sets of 100 painted RIFA workers had each been allowed to feed upon the bait for 5 h, the bait was removed and each set of 100 ants was immediately placed into one of the 18 previously mentioned plastic shoe boxes containing RIFA that had not been fed for 2 d. Of the 12 sets of 100 painted RIFA that had fed upon Calco blue-dyed 0.045% Advion™, six sets were placed into the six plastic shoe boxes containing 1000 workers each that comprised Treatment 1, and the remaining six sets were placed into the six plastic shoe boxes containing 1000 workers and ~250 brood each that comprised Treatment 2. Of the six sets of 100 painted RIFA that had fed upon Calco blue-dyed 0.0% Advion™, three sets were placed into the three plastic shoe boxes containing 1000 workers each that comprised Control 1, and the remaining three sets were placed into the three plastic shoe boxes containing 1000 workers and ~250 brood each that comprised Control 2. Therefore, at this point, each replicate of Treatment 1 consisted of 1100

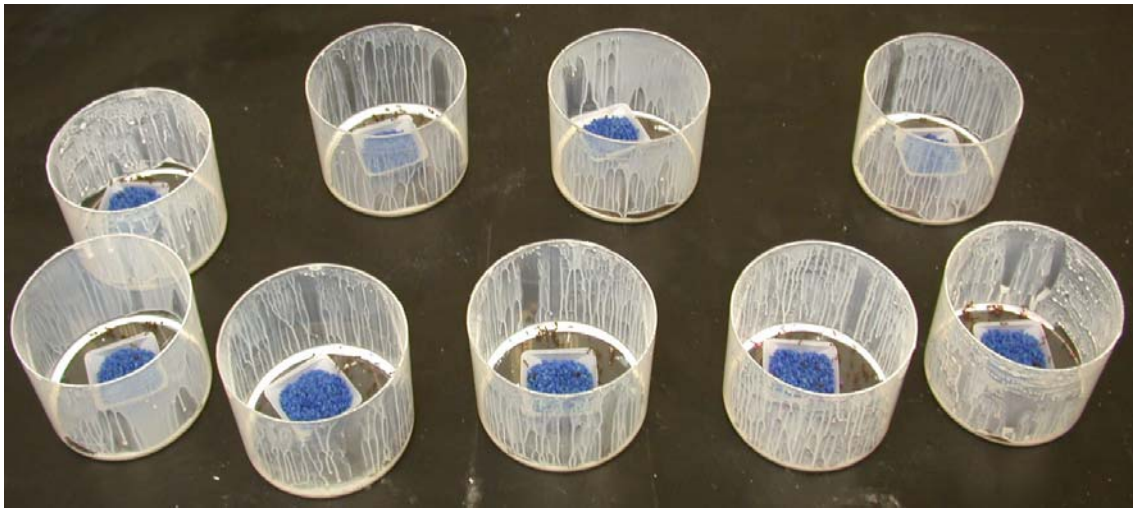

Figure 13. A group of Fluon®-lined, 9.5 cm x 6.5 cm plastic cylindrical containers used for the experiment on trophallactic transmission and metabolism of the active ingredient indoxacarb in Advion™. Each plastic cylinder contained a set of 100 fluorescent pink painted RIFA workers, and a single 4 cm x 0.8 cm weigh dish filled with either 1 g of Calco blue-dyed 0.045% Advion™ or 1 g of Calco blue-dyed 0.0% Advion™ (contained no indoxacarb).

RIFA workers, each replicate of Treatment 2 consisted of 1100 RIFA workers and ~250 brood, each replicate of Control 1 consisted of 1100 RIFA workers, and each replicate of Control 2 consisted of 1100 RIFA workers and ~250 brood.

After 24 hours had passed since the 18 sets of 100 painted RIFA workers had been introduced into the appropriate replicates, all RIFA workers within three of the replicates for Treatment 1 and 3 of the replicates for Treatment 2 were divided into two groups: those RIFA workers that were dead and those RIFA workers that were still living. The dead RIFA workers were removed from each of these six plastic shoe boxes, and temporarily stored in separate 9 cm x 1.5 cm petri dishes that were numbered to

correspond with the replicate from which the ants had been removed. Each of the six plastic shoe boxes was then placed in a GE® model FUM 21DAARWH freezer (General Electric Company, Fairfield, CT) for 2 h at less than -20° C to kill all living RIFA workers, so as to ensure that no further trophallactic activity occurred among the ants.

The shoe boxes were removed from the freezer after 2 h, and the RIFA workers within each shoe box were individually crushed in a manner similar to that described by Summerlin et al. (1975). First, a single RIFA worker was placed into each of 100 grid squares on a piece of white 21.6 cm x 27.9 cm grid paper. For statistical purposes, a Lights of America® model 7020 black light (Lights of America, Inc., Walnut, CA) was then used to determine if any of the RIFA workers on a given sheet of grid paper were marked with fluorescent-pink paint, thus identifying the painted RIFA workers that had originally fed upon the Calco blue-dyed 0.045% Advion™. Though common data sets were produced for all RIFA workers in this experiment, painted workers were identified in the aforementioned manner in order to produce separate data sets for the painted RIFA workers, as well.

Then, once a sheet of grid paper had been filled and scanned with the black light for the presence of painted ants, each of the RIFA workers on the grid paper was individually crushed using a sterile Eppendorf® micropestle. Sets of 100 Eppendorf® micropestles were used and, following use, each set was washed in soapy water and soaked in alcohol for 30 min. This was done to ensure that no Calco blue dye was present on any of the micropestles prior to being re-used, so as to prevent false positive readings. After an individual ant was crushed, the grid square was observed to

determine if blue dye visibly stained the grid square (Figure 14). If any amount of blue dye was seen, then the RIFA worker was determined to have contained the Calco blue-dyed, indoxacarb-containing oil with which the corncob based grit of the bait had been saturated. In this manner, all of the 1100 RIFA workers within each of the six plastic shoe boxes that were selected for this portion of the experiment were crushed, revealing the presence or absence of Calco blue-dyed oil and, thus, the presence or absence of indoxacarb. Resultantly, multiple data sets of mortality and survivorship of the RIFA workers used in this experiment, as well as the number of RIFA workers within these data sets that were found to contain Calco blue dye, were produced.

The brood from the three replicates that consisted of both RIFA workers and brood in which all RIFA workers were crushed were also examined. Crushing the brood in the same manner as the workers to determine the presence or absence of Calco blue dye was not feasible, however, as pre-experiment testing of that method on the brood resulted in numerous false-negative tests for the presence of Calco blue dye due to the soft bodies of the larvae. Therefore, after all RIFA workers had been removed from the artificial brood chambers within the three previously mentioned replicates, the brood inside of each individual brood chamber were viewed en masse under an American Optical® Stereo Star® 0.7x to 4.2x binocular dissecting microscope, model #AO 570 (American Optical, San Diego, CA), to determine the number of translucent larvae that contained Calco blue dye.

Figure 14. White 21.6 cm x 27.9 cm grid paper that was used for the experiment on trophallactic transmission and metabolism of the active ingredient indoxacarb in Advion™. A single RIFA worker was placed into each of 100 grid squares on the paper, and each ant was individually crushed with an Eppendorf® micropestle in order to reveal the presence (+) or absence of Calco blue dye within the ant.

The remaining three replicates of Treatment 1, the remaining three replicates of Treatment 2, the three replicates of Control 1, and the three replicates of Control 2, were all observed 24 h after the introduction of the sets of 100 painted RIFA workers in order to estimate percent RIFA worker mortality. In like fashion, each of these replicates was observed every 24 h thereafter for the duration of the 30 d experiment. Resultantly, data sets of daily cumulative RIFA worker mortality were produced, allowing for comparison

of indoxacarb-induced mortality between Treatment 1, consisting of only RIFA workers, and Treatment 2, consisting of both RIFA workers and brood.

Additionally, tests were conducted to determine if RIFA workers were adversely affected by ingesting the Calco blue dye contained in the Advion™ utilized in the previously mentioned experiment, or by being painted with fluorescent spray paint. Test 1 evaluated the effects of RIFA workers ingesting Calco blue-dye. For this test, there was a single treatment group and a single control group, each consisting of three replicates. Each replicate consisted of 100 RIFA workers, all of which were aspirated from the same colony that was used in the previously mentioned experiment. Each set of 100 RIFA workers was placed into a separate 9.5 cm x 6.5 cm Fluon®-lined plastic cylindrical container. For the three replicates of the treatment group, each plastic cylinder contained a 4 cm x 0.8 cm weigh dish with 1 g of Calco blue-dyed 0.0% Advion™, and a 4 cm x 0.8 cm weigh dish filled with water-soaked cotton. For the 3 replicates of the control group, each plastic cylinder contained a 4 cm x 0.8 cm weigh dish with 1 g of un-dyed 0.0% Advion™, and a 4 cm x 0.8 cm weigh dish filled with water-soaked cotton. All RIFA in both groups were allowed to feed on their respective bait for the duration of the 30 d test, which was conducted concurrently with the previously described experiment, and mortality was recorded once daily.

Test 2 evaluated the effects of painting RIFA workers with fluorescent spray paint. As with the previous test, there was a single treatment group and a single control group, each consisting of three replicates. Each replicate consisted of 100 RIFA workers, all of which were aspirated from the same colony that was used in the previously mentioned

experiment. For the treatment group, each of the three sets of 100 RIFA workers was first spray painted with Krylon® Fluorescent Pink Indoor/Outdoor Paint, in a manner identical to that described in the previously mentioned experiment. The three sets of 100 painted RIFA workers were then placed into separate 9.5 cm x 6.5 cm Fluon®-lined plastic cylindrical containers, each of which contained a 4 cm x 0.8 cm weigh dish with 1 g of Calco blue-dyed 0.0% Advion™, and a 4 cm x 0.8 cm weigh dish filled with water-soaked cotton. For the control group, the RIFA workers were left unpainted, and the three sets of 100 unpainted RIFA workers were simply placed into separate 9.5 cm x 6.5 cm Fluon®-lined plastic cylindrical containers. As with the treatment group, each plastic cylinder contained a 4 cm x 0.8 cm weigh dish with 1 g of Calco blue-dyed 0.0% Advion™, and a 4 cm x 0.8 cm weigh dish filled with water-soaked cotton. All RIFA in both groups were allowed to feed on the bait for the duration of the 30 d test, which was conducted concurrently with the previously described experiment, and mortality was recorded once daily.

Statistics. At the conclusion of this experiment, SPSS® software (SPSS 2001) was used to conduct statistical analysis of the data. For the tests that evaluated the effect of RIFA workers feeding on Calco blue-dyed Advion™ and the effect of RIFA workers being painted with fluorescent spray paint, ANOVA was first conducted on the mortality data sets that were produced from these tests. Then, the LSD post hoc test was conducted on the mortality data sets to determine significant differences among the treatment and control groups. All tests of significance were evaluated at $P = 0.05$.

In terms of the six total replicates of Treatments 1 and 2 in which all RIFA workers were crushed, ANOVA was first conducted on the survivorship and mortality data sets produced from this portion of the experiment, and the LSD post hoc test was then conducted on those same data sets to determine significant differences between the treatments. All RIFA workers, both those that were originally painted with fluorescent spray paint and those that were not, were included in the aforementioned statistical analysis. Additionally, separate statistical analysis was conducted on the survivorship and mortality data sets of the RIFA workers that had been painted with fluorescent spray paint. As with the previous data sets, both ANOVA and the LSD post hoc test were conducted on the survivorship and mortality data sets of the painted RIFA workers. All tests of significance were evaluated at $P = 0.05$.

With relation to the remaining six total replicates of Treatments 1 and 2 and the six total replicates of Controls 1 and 2 in which the percent of cumulative RIFA worker mortality was determined each day for the duration of the experiment, Abbott's formula was first used to correct the mortality data sets that resulted from this portion of the experiment (Abbott 1925). Abbott's formula for correcting mortality data is

$$[(X-Y) / X] \times 100 = \text{percent control}$$

where "X" is the percent survival in the control group, and "Y" is the percent survival in the treatment group. Next, ANOVA was conducted on the corrected mortality data sets and, finally, the LSD post hoc test was conducted on those same data sets to determine

significant differences among treatments. All tests of significance were evaluated at P = 0.05.

Results

Cumulative percent RIFA worker mortality for the tests that were conducted to evaluate the effects of RIFA workers ingesting Calco blue dye and the effects of painting RIFA workers with fluorescent spray paint are shown in Table 24 and Figure 15 ($F=1.77$; $df=119$; $P<0.05$). When comparing the treatment group in Test 1, RIFA workers that were fed Calco blue-dyed 0.0% Advion™, to the control group in Test 1, RIFA workers that were fed un-dyed 0.0% Advion™, there was no significant difference ($P>0.05$) in RIFA worker mortality for any of the 30 d during which this test was conducted. Similarly, when comparing the treatment group in Test 2, painted RIFA workers that were fed Calco blue-dyed 0.0% Advion™, to the control group in Test 2, unpainted RIFA workers that were fed Calco blue-dyed 0.0% Advion™, there was no significant difference ($P>0.05$) in RIFA worker mortality for any of the 30 d. Furthermore, when comparing the treatment and control group of Test 1 to the treatment and control group of Test 2, there was no significant difference ($P>0.05$) for any of the 30 d.

Mean percentages of all RIFA workers that contained blue dye 24 h after sets of 100 fluorescent painted RIFA workers that had been treated with Calco blue-dyed 0.045% Advion™ were introduced into replicates that contained 1000 unfed RIFA workers, or into replicates that contained 1000 unfed RIFA workers and ~250 brood, are shown in

Table 24. Comparison of the mean percent mortality for a test evaluating the effects of RIFA workers ingesting Calco blue-dye (Test 1) and a test evaluating the effects of painting RIFA workers with fluorescent spray paint (Test 2).

Day	Mean cumulative percent RIFA worker mortality per treatment[a] (mean ± SD)			
	Test 1 treatment- RIFA fed Calco blue-dyed 0.0% Advion™	Test 1 control- RIFA fed un-dyed 0.0% Advion™	Test 2 treatment- painted RIFA fed Calco blue-dyed 0.0% Advion™	Test 2 control- unpainted RIFA fed Calco blue-dyed 0.0% Advion™
1	2.0±0.0a	1.0±1.7a	1.7±0.6a	0.7±1.2a
2	2.3±0.6a	2.3±1.2a	2.7±1.2a	1.7±0.6a
3	3.0±1.0a	2.7±1.5a	3.0±1.0a	2.7±0.6a
4	3.7±0.6a	3.3±1.2a	3.7±1.2a	3.7±2.1a
5	4.3±1.2a	3.7±1.5a	5.0±1.0a	5.0±1.7a
6	4.7±0.6a	4.7±0.6a	6.3±1.5a	5.7±1.2a
7	5.7±0.6a	5.0±1.0a	7.0±1.0a	6.3±1.5a
8	6.3±1.2a	5.3±1.2a	7.3±1.5a	7.0±1.0a
9	7.7±0.6a	6.3±1.5a	8.0±1.0a	7.0±1.0a
10	8.3±0.6a	7.3±1.5a	9.0±1.0a	8.3±1.2a
11	9.3±0.6a	8.7±2.1a	10.0±1.0a	9.0±1.0a
12	10.0±1.0a	9.7±2.1a	11.0±1.7a	9.3±0.6a
13	10.7±0.6a	10.7±2.1a	11.7±1.5a	11.0±1.0a
14	11.3±1.2a	11.3±1.5a	12.3±1.2a	11.7±2.1a
15	12.0±1.0a	12.0±2.0a	13.7±1.5a	12.7±2.1a
16	13.3±1.5a	13.0±2.0a	14.0±2.0a	13.0±2.6a
17	14.3±1.5a	14.0±1.7a	15.0±1.0a	15.0±2.6a
18	15.7±2.1a	14.7±1.5a	16.0±1.7a	15.7±2.1a
19	17.3±1.5a	16.3±1.5a	16.7±1.5a	16.7±2.9a
20	18.3±1.5a	16.7±2.1a	18.7±2.5a	18.0±2.6a
21	19.3±1.5a	18.0±2.0a	20.3±3.2a	19.3±3.2a
22	20.7±0.6a	19.7±2.9a	20.7±3.1a	21.0±2.6a
23	23.0±1.0a	20.7±2.1a	21.7±3.1a	22.0±2.6a
24	25.0±1.0a	21.7±3.8a	23.0±2.6a	23.0±2.6a
25	25.7±1.2a	23.7±2.5a	24.3±2.5a	24.0±3.5a
26	26.7±1.2a	24.0±3.0a	25.7±2.1a	25.3±3.1a
27	27.7±1.5a	25.3±1.5a	27.3±3.5a	27.0±2.0a
28	28.3±0.6a	26.0±2.0a	28.7±3.1a	27.7±2.1a
29	30.0±1.0a	27.7±2.5a	30.0±1.7a	30.0±2.6a
30	32.0±1.0a	29.7±1.2a	31.7±3.1a	32.0±3.5a

[a] Means within a row with different letters are significantly different at P<0.05. Means were separated using the LSD test.

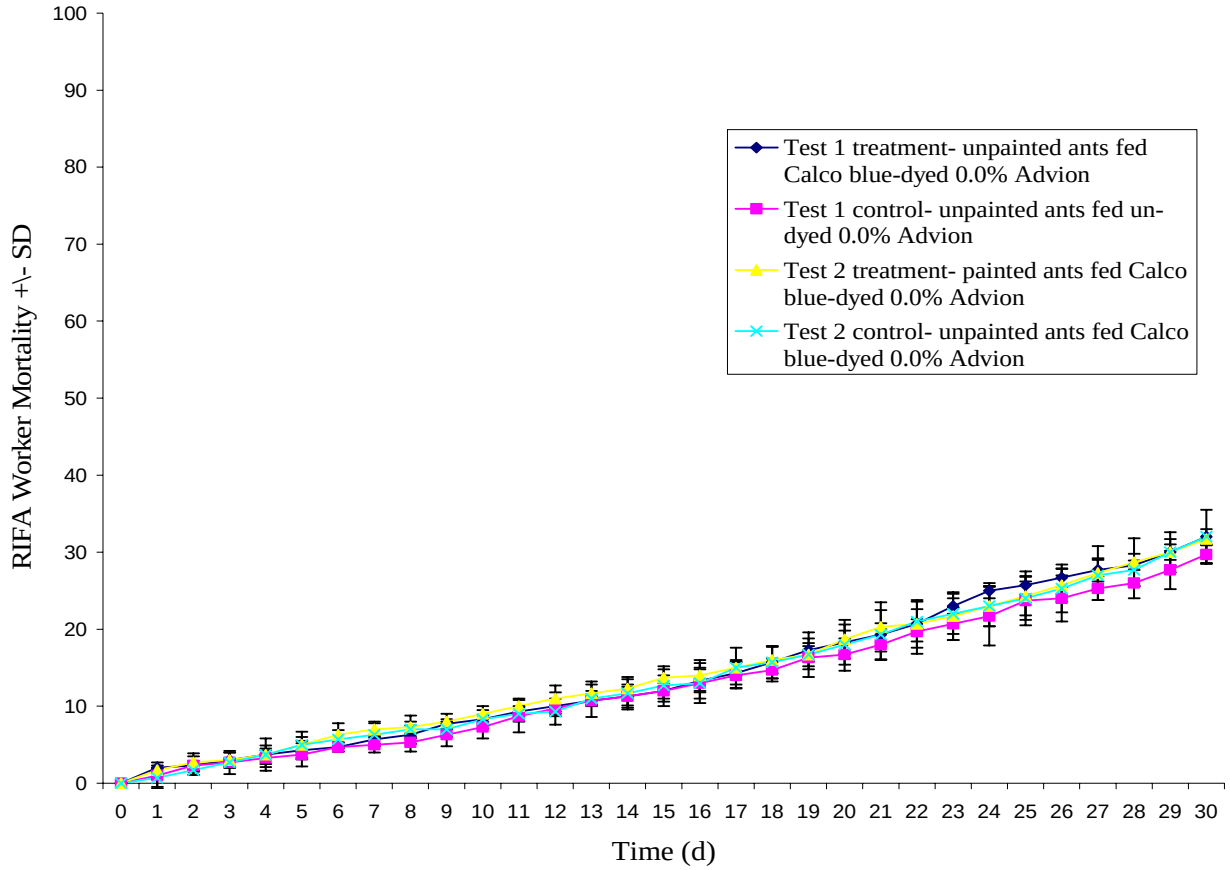

Figure 15. A comparison of the mean percent mortality for a test evaluating the effects of RIFA workers ingesting Calco blue-dye (Test 1) and a test evaluating the effects of painting RIFA workers with fluorescent spray paint (Test 2).

Table 25. At least 50% of all RIFA workers in both the replicates containing RIFA workers only and the replicates containing RIFA workers and brood contained Calco blue dye, though a significantly greater number ($P<0.05$) of RIFA workers in the replicates of the former treatment contained Calco blue dye. There was, however, no significant difference ($P>0.05$) between treatments in terms of the mean number of dead RIFA workers, with the mean ranging from 23.9% to 26.3%. Of those dead RIFA workers, >77% contained Calco blue dye, and there was no significant difference ($P>0.05$) between treatments. Similarly, no significant difference ($P>0.05$) existed between treatments in terms of the mean number of living RIFA workers, as the mean ranged from 73.7% to 76.1%. However, a significantly greater ($P<0.05$) number of living RIFA workers contained Calco blue dye in the replicates containing workers only (45.6%) than in the replicates containing both workers and brood (41.3%).

Mean percentages of fluorescent-painted RIFA workers that contained blue dye 24 h after sets of 100 fluorescent painted RIFA workers that had been treated with Calco blue-dyed 0.045% Advion™ were introduced into replicates that contained 1000 unfed RIFA workers, or into replicates that contained 1000 unfed RIFA workers and ~250 brood, are shown in Table 26. There was no significant difference ($P>0.05$) between treatments for any of the means that were compared. At least 71% of the painted RIFA workers in both the replicates containing RIFA workers only and the replicates containing RIFA workers and brood contained Calco blue dye. Between 72% and 75% of the painted RIFA workers were dead, and of those >90% contained Calco blue dye.

Table 25. Mean percentages of all RIFA workers that contained blue dye 24 h after sets of 100 fluorescent painted RIFA workers that had been treated with Calco blue-dyed 0.045% Advion™ were introduced into replicates that contained 1000 unfed RIFA workers (Treatment 1), or into replicates that contained 1000 unfed RIFA workers and ~250 brood (Treatment 2). All RIFA workers were individually crushed on white grid paper in order to determine the presence or absence of blue dye within each worker. Prior to being crushed, the RIFA workers were separated into two groups, living or dead, for statistical purposes.

Mean percentages	Mean percentages of RIFA workers containing Calco blue dye 24 h after Advion™-treated RIFA workers were introduced into the replicates, per treatment[a] (mean ± SD)		F	P
	Treatment 1- workers only (no brood)	Treatment 2- workers and brood		
RIFA workers, dead or living, containing blue dye	54.8±2.0a	49.9±2.0b	9.16	0.04
Dead RIFA workers	26.3±3.1a	23.9±1.7a	1.50	0.29
Dead RIFA workers containing blue dye	80.5±2.3a	77.4±2.0a	3.09	0.15
Living RIFA workers	73.7±3.1a	76.1±1.7a	1.50	0.29
Living RIFA workers containing blue dye	45.6±1.3a	41.3±1.9b	11.09	0.03

[a] Means within a row with different letters are significantly different at $P<0.05$. Means were separated using the LSD test.

Table 26. Mean percentages of fluorescent-painted RIFA workers that contained blue dye 24 h after sets of 100 fluorescent painted RIFA workers that had been treated with Calco blue-dyed 0.045% Advion™ were introduced into replicates that contained 1000 unfed RIFA workers (Treatment 1), or into replicates that contained 1000 unfed RIFA workers and ~250 brood (Treatment 2). All RIFA workers were individually crushed on white grid paper in order to determine the presence or absence of blue dye within each worker. Prior to being crushed, the RIFA workers were separated into two groups, living or dead, for statistical purposes.

Mean percentages	Mean percentages of fluorescent painted, Advion™-treated RIFA workers containing Calco blue dye 24 h after being introduced into the replicates, per treatment[a] (mean ± SD)		F	P
	Treatment 1- workers only (no brood)	Treatment 2- workers and brood		
Fluorescent painted RIFA workers, dead or living, containing blue dye	71.0±2.6a	72.7±2.5a	0.63	0.47
Dead fluorescent painted RIFA workers	72.0±5.3a	75.0±5.6a	0.46	0.54
Dead fluorescent painted RIFA workers containing blue dye	90.3±2.5a	90.8±2.9a	0.01	0.98
Living fluorescent painted RIFA workers	28.0±5.3a	25.0±5.6a	0.46	0.54
Living fluorescent painted RIFA workers containing blue dye	19.9±4.1a	18.9±2.5a	0.11	0.76

[a] Means within a row with different letters are significantly different at $P<0.05$. Means were separated using the LSD test.

Between 25% and 28% of the painted RIFA workers were living, and of those ~19-20% contained Calco blue dye.

In terms of the brood that were observed from the three replicates that contained both RIFA workers and brood in which all RIFA workers were crushed, less than five larvae per artificial brood chamber contained Calco blue dye. It was not possible to perform statistics on these data, as the number of larvae in each artificial brood chamber was highly variable, resulting from the fact that the brood in each replicate were determined by weight and not by an exact number of each brood stage. Furthermore, not all larvae were translucent at the time of viewing, preventing determination of the presence or absence of Calco blue dye within those larvae.

A comparison of the mean percent RIFA worker mortality resulting from treatment with 0.045% Advion™ in replicates that contained 1100 RIFA workers, and in replicates that contained 1100 RIFA workers and ~250 brood, is shown in Table 27 and Figure 16 ($F=3.01$; $df=59$; $P<0.05$). The only time in which there was a significant difference in RIFA worker mortality between treatments were days 2 and 3, when there was significantly greater mortality ($P<0.05$) in the replicates that contained RIFA workers only. From day 4 through the end of the experiment, there was no significant difference ($P>0.05$) in RIFA worker mortality between treatments.

Table 27. Comparison of the mean percent RIFA worker mortality resulting from treatment with 0.045% Advion™ in replicates that contained 1100 RIFA workers (Treatment 1), and in replicates that contained 1100 RIFA workers and ~250 brood. (Treatment 2).

	Mean cumulative percent RIFA worker mortality per treatment[a] (mean ± SD)	
Day	Treatment 1- workers only (no brood)	Treatment 2- workers and brood
1	26.2±3.1a	24.2±5.0a
2	52.6±2.9a	47.1±2.9b
3	67.0±2.9a	60.1±2.9b
4	76.7±2.7a	73.4±1.0a
5	80.7±2.1a	78.2±1.2a
6	81.4±1.5a	80.2±1.2a
7	82.4±1.0a	81.5±1.6a
8	82.6±0.8a	82.3±1.4a
9	83.6±0.4a	82.3±1.4a
10	83.6±0.4a	82.7±1.1a
11	84.3±0.4a	83.4±1.1a
12	84.3±0.4a	83.4±1.1a
13	84.5±0.5a	84.4±1.1a
14	84.6±0.4a	84.4±1.1a
15	84.6±0.4a	84.4±1.1a

[a] Mortality was corrected by the formula in Abbott (1925). Means within a row with different letters are significantly different at $P<0.05$. Means were separated using the LSD test.

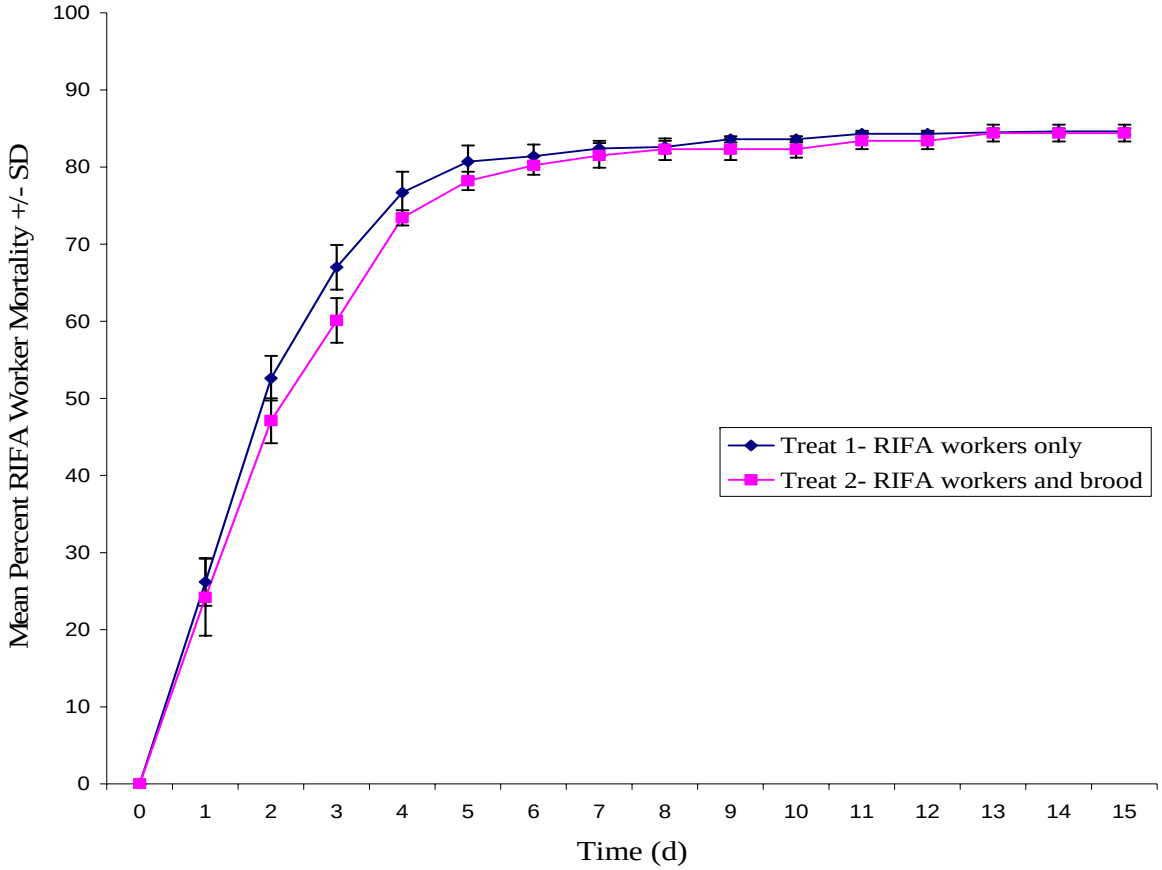

Figure 16. A comparison of the mean percent RIFA worker mortality resulting from treatment with 0.045% Advion™ in replicates that contained 1100 RIFA workers (Treatment 1), and in replicates that contained 1100 RIFA workers and ~250 brood (Treatment 2).

Discussion and Conclusions

One of the unique aspects of indoxacarb, the active ingredient in the new RIFA bait Advion™, is that it must first be metabolized by the insect in order to become acutely toxic. The metabolism of indoxacarb into the toxic JT333 has been well-studied in certain insects (Wing et al. 1998, 2000). In terms of metabolism of indoxacarb by the RIFA, it is commonly believed that within a RIFA colony the larvae are responsible for metabolizing indoxacarb (E.I. DuPont de Nemours and Company 2004). However, metabolism of indoxacarb has not been well-studied in the RIFA worker caste. Furthermore, for the active ingredient in a RIFA bait to be effective, it must be passed through the colony via the process of trophallaxis (Collins and Callcott 1998). Though it is assumed that indoxacarb is passed through a RIFA colony via trophallaxis, no formal experimentation has been conducted to visually demonstrate this process. Utilizing Advion™ as the source of indoxacarb, this research was conducted for the aforementioned reasons, thus producing data that both demonstrated the trophallactic transmission of indoxacarb among the RIFA and determined if RIFA workers were capable of metabolizing indoxacarb into JT333.

Prior to reaching conclusions based on the results of this research, it is first necessary to discuss the findings of the two tests that were conducted to determine if RIFA workers were adversely affected by ingesting Calco blue dye or by being painted with fluorescent spray paint. The data in Table 24 revealed that there was no significant difference ($P>0.05$) in RIFA worker mortality between workers that were fed Calco blue-dyed 0.0% Advion™ and workers that were fed un-dyed 0.0% Advion™, indicating that

ingestion of the Calco blue dye had no adverse effects on RIFA workers. The data in Table 24 also revealed that there was no significant difference ($P>0.05$) in RIFA worker mortality between workers that were painted with fluorescent spray paint and those that were not painted, indicating that the fluorescent spray paint had no adverse effects on RIFA workers. Furthermore, there was no significant difference ($P>0.05$) in RIFA worker mortality among any of these four groups, fortifying the conclusion that neither the Calco blue dye nor the fluorescent spray paint had any adverse effects on the RIFA workers in this experiment.

In terms of trophallactic transmission of indoxacarb, the results of this experiment indicated that indoxacarb, delivered via Advion™, was passed among the RIFA by the process of trophallaxis. This conclusion was based on the data found in Table 25, which revealed that only 24 h after Calco blue-dyed Advion™-fed workers had been introduced into replicates containing workers only or both workers and brood, ~50-55% of all RIFA workers contained Calco blue dye. Since the 1000 unfed workers within each replicate were never allowed to feed directly upon the Advion™ granules, the only way that they could have acquired the Calco blue dye was via trophallaxis from the 100 Calco blue-dyed Advion™-fed workers that were introduced into each replicate. Though the numbers were very small, trophallactic transmission of indoxacarb was also demonstrated by the fact that RIFA larvae in each of the three replicates containing both RIFA workers and brood in which all RIFA workers were crushed contained Calco blue dye. Although less than five larvae per replicate were determined to have contained

Calco blue dye, the fact that any of them contained blue dye could only have been the result of trophallaxis.

It is important to understand that the presence of Calco blue dye within the RIFA in this experiment indicated the presence of indoxacarb. The reason for this lies in how the Calco blue-dyed Advion™ is produced. Both the oil soluble Calco blue dye and the indoxacarb are initially added to soybean oil, which is then applied to the grit. The soybean oil, which is the attractant in this and most RIFA baits, is what the RIFA were actually passing to one another via trophallaxis. As ants received the soybean oil from other ants, they were also receiving the other two substances that had been solubilized within the soybean oil: Calco blue dye and indoxacarb. Therefore, the presence of Calco blue dye within the RIFA in this experiment indicated the presence of the soybean oil within those same ants and, thus, the presence of indoxacarb.

The fact that the Calco blue dye indicated the presence of indoxacarb was supported by the data in Table 25. Of the ~24-26% of RIFA workers that were found dead 24 h after the introduction of the 100 Calco blue-dyed Advion™-fed workers into replicates containing workers only or both workers and brood, ~77-80% of them contained Calco blue dye. Further, the data in Table 26 revealed that of the 72-75% of Calco blue-dyed Advion™-fed RIFA workers that were dead 24 h after their introduction into replicates containing workers only or both workers and brood, ~90% of them contained Calco blue dye. These data offered strong evidence in support of the fact that Calco blue dye was an indicator of the presence of indoxacarb, resulting from the previously mentioned process in which the Calco blue-dyed Advion™ was produced. Therefore, the results of

this experiment indicated that indoxacarb, delivered via Advion™, was passed among the RIFA by the process of trophallaxis.

In terms of the ability of RIFA workers to metabolize indoxacarb, the results of this research revealed that workers were indeed capable of metabolizing indoxacarb into the toxic JT333. The data in Table 27 revealed that there was no significant difference ($P>0.05$) in RIFA worker mortality between the treatment consisting of RIFA workers only and the treatment consisting of both RIFA workers and brood for all but 2 d of the experiment. These data, paired with the fact that RIFA worker mortality ultimately reached >85% in the absence of brood, indicated that the workers were capable of metabolizing indoxacarb into the toxic metabolite. This conclusion was further reinforced by the data in Table 26. These data indicated that out of the ~72% of Calco blue-dyed Advion™-fed RIFA workers that were dead 24 h after their introduction into replicates containing workers only, ~90% of them contained Calco blue dye. Furthermore, Table 25 revealed that of the ~26% of RIFA workers that were found dead 24 h after the introduction of the 100 Calco blue-dyed Advion™-fed workers into replicates containing workers only, ~80% of them contained Calco blue dye. Since Calco blue dye was an indicator of the presence of indoxacarb, as previously discussed, these two additional pieces of data supported the conclusion that RIFA workers were capable of metabolizing indoxacarb into JT333 by providing evidence that indoxacarb had in fact been ingested by the ants upon which the previously mentioned statistics were based.

In conclusion, the data indicated that neither the Calco blue dye nor the fluorescent spray paint used in this experiment had any adverse effect on RIFA workers. Having established that, the results of this experiment revealed that indoxacarb delivered via Advion™ was passed among the RIFA via the process of trophallaxis, as demonstrated by both RIFA workers and RIFA larvae. And, finally, the data from this research clearly indicated that RIFA workers were capable of metabolizing indoxacarb into JT333.

As with all research, duplication is necessary to further validate these conclusions, and future research should ideally include more than three replicates. Future research should also attempt to determine the number of RIFA workers and larvae containing Calco blue dye by crushing all workers and microscopically examining all larvae in selected replicates after various amounts of time, such as after 48 h and 72 h. This would provide a set of data detailing the flow of indoxacarb through the workers and larvae within a RIFA colony over a clearly defined period of time. Additionally, for the replicates that were observed each day for 30 d, dead RIFA workers should ideally be removed each day and crushed to determine the presence or absence of Calco blue dye within the dead workers. This would provide further statistical evidence in support of the correlation between the presence of Calco blue dye and the presence of indoxacarb within RIFA workers.

CHAPTER VI

THE EFFECTIVENESS OF LABEL-RATE BROADCAST TREATMENT WITH ADVION™ AT CONTROLLING MULTIPLE ANT SPECIES

Introduction

A wide variety of chemicals and methods of delivering those chemicals have been utilized in an attempt to control the red imported fire ant (RIFA), *Solenopsis invicta* Buren, since its accidental introduction into the United States in the 1930's (Banks 1990; Collins et al. 1992; Eden and Arant 1949; Lofgren et al. 1964; Lofgren et al. 1975; Phillips and Thorvilson 1989; Sauer et al. 1982; Vander Meer et al. 1982). Beginning in the 1960's and 1970's, the effectiveness of baits as a method of chemical delivery was realized, and their use became increasingly popular both for the control of the RIFA and for the control of other ant species. Baits were very effective because they took advantage of the complex, trophallaxis-driven, social feeding behavior exhibited by the RIFA and numerous other ant species. These popular baits were commonly composed of corncob grit-based granules, which were coated with some type of oil (most commonly soybean oil) to which the insecticide had been added (Banks 1990; Lofgren et al. 1964). Essentially, this same recipe for baits is still used today, and only the active ingredient has changed.

Advion™ (E.I. DuPont de Nemours and Company, Wilmington, DE) is one of the newest corncob grit-based baits developed for the control of the RIFA. Advion™,

which received approval from the United States Environmental Protection Agency in August of 2004 (EPA Registration #352-627), contains the active ingredient indoxacarb, a pyrazoline-type insecticide that is classified as an oxadiazine. Discovered by E.I. DuPont de Nemours and Company in 1991, the chemical indoxacarb is a novel insecticide that must first be metabolized in order to become acutely toxic. Following ingestion by the insect, metabolic breakdown of indoxacarb occurs via amidase and esterase enzymes commonly found within the midgut and/or fat bodies, ultimately producing an N-decarbomethoxyllated metabolite known as JT333. It is JT333 that is so highly toxic to the insect, functioning as a potent, voltage-dependent sodium channel blocker. The bio-activation of this potent metabolite within the insect ultimately results in severe neurotoxic symptoms, paralysis, and a relatively rapid death (Wing et al. 2000).

As with many RIFA baits, Advion™ can either be applied as a broadcast treatment or utilized as an individual mound treatment. While both application methods have the potential to effectively achieve control of the target species, individual mound treatments are generally more expensive and more labor intensive than broadcast treatments due to the fact that a greater amount of bait is usually necessary to attain equivalent control and the fact that each mound in an area must be treated individually (Barr 1999; Barr and Best 1999; Barr et al. 1999; Drees et al. 1996; Vail 1998). However, one advantage of individual mound treatments is that they are directed at controlling a single species. This can be an important issue, as it is commonly the case that numerous ant species other than the target species are also attracted to and affected by the bait. Therefore, by

limiting application of the bait to the area directly around the mound, it is possible to prevent non-target ant species from being effected by the treatment. Conversely, broadcast treatments potentially affect numerous ant species in the area that has been treated. Therefore, if the goal is to achieve control of numerous species of ants in an area, broadcast treatment is more likely to be effective (Drees et al. 1996).

Though research has been conducted to determine the effectiveness of broadcast treatment with Advion™ at controlling the RIFA, no research has previously been conducted or published in an attempt to determine the effectiveness of such treatment at controlling ant species other than the RIFA. Resultantly, the objective of this research was to evaluate the effectiveness of label-rate broadcast treatment with Advion™ at controlling multiple ant species. Specifically, in addition to the RIFA, this research evaluated the effects of Advion™ on *Monomorium pharaonis*, *Pogonomyrmex barbatus*, and *Dorymyrmex pyramicus*, all of which inhabited the common land area utilized for this research. *M. pharaonis*, *P. barbatus*, and *D. pyramicus* are all commonly considered to be pest species and, therefore, control measures are regularly implemented in an attempt to manage these ants (Ebeling 1975; Haack and Granovsky 1990; Harwood and James 1979; Nickerson et al. 2003). Resultantly, this research attempted to address the existing gap in knowledge concerning the effects of Advion™ on multiple ant species by evaluating the effectiveness of label-rate broadcast treatment with Advion™ at controlling *M. pharaonis*, *P. barbatus*, and *D. pyramicus*, in addition to the RIFA.

Materials and Methods

This experiment was conducted in the field on a private game ranch (N28°05’79” W98°05’75”) located in Jim Wells County, Texas. The property was ~4856 ha, with the majority of the land groomed for dove, quail and deer hunting. A considerable portion of the land was utilized for cattle grazing, as well. Though there were a variety of different types of vegetation throughout the property, this experiment was conducted on a 2 ha field consisting of grass pastureland. No cattle grazing was allowed on this field immediately preceding the experiment, during the experiment, or immediately following the experiment. The grass comprising this 2 ha field was mowed ~1 wk prior to the beginning of the experiment, and then approximately every 2 wk thereafter, though the grass was never mowed within 1 wk prior to any of the ant surveys that were conducted over the course of the study.

Prior to beginning the experiment, 20 rectangular shaped 0.04 ha plots were measured via the use of a Rolatape® M300 series measuring wheel (Rolatape Corp., Spokane, WA). Each plot was separated from the next nearest plot by at least 15 m. A 46 cm x 5 cm x 2 cm wooden stake was then hammered into the soil at each of the four corners of a given plot, and #16 polyester/cotton twine was used to connect one stake to the next, thus forming a clearly demarcated rectangular border. The next day, which was the day immediately preceding treatment of the plots, a survey of ant species was conducted by placing individually numbered and baited Fisherbrand® 1.7 cm x 6.0 cm threaded glass 8 ml vials (Fisher Scientific International Inc., Hampton, NH) within the border of each plot. Each vial contained either a protein-based food source or a carbohydrate-based

food source as bait. The protein-based food source was canned tuna fish packed in water (Weeks et al. 2004). After being drained, ~1.5 g of tuna was placed into each of 100 vials. The carbohydrate-based food source consisted of a 60% sugar water solution (Weeks et al. 2004). Cotton plugs were soaked in the 60% sugar water solution, and then a single plug was placed into each of 100 vials. All baited vials were prepared in this manner on the morning in which they were placed out in the field. On June 3, 2004, the morning in which this initial pre-treatment survey was conducted, a total of 10 baited vials (five vials baited with tuna and five vials baited with sugar water solution) were individually placed out in linear fashion at 2-3 m intervals within each plot, with the vials being placed on their sides to allow ants easy access to the bait. Dispersal of the baited vials began at 9 a.m. C.S.T., and collection of the vials began at 10 a.m. C.S.T., thus allowing the ants 1 h to locate and feed upon the food source within each vial (Lemke and Kissam 1988). Vials were capped upon collection and taken to a laboratory setting, where species identification and estimation of the number of each species of ant per vial were determined.

For this experiment, there was a single treatment group and a control group. The treatment group consisted of plots subjected to label-rate broadcast treatment with 0.045% Advion™, and the control group consisted of plots that were left untreated. There were 10 replicates each for both the treatment group and the control group, thus a total of 20 plots were utilized for this experiment. Experimentation was conducted for a total of 7 wk, from June 4 through July 23, 2004. Between approximately 9:00 a.m. and 10:00 a.m. C.S.T. on the first morning of the experiment, the plots in the treatment group

were subjected to broadcast treatment with 0.045% Advion™ at label rate (1.7 kg/ha) via the use of a Scotts® Handy Green II® hand spreader (Scotts Company, Marysville, OH).

The next morning, in a manner identical to that of the aforementioned pre-treatment ant survey, a total of 10 individually numbered and baited vials (five containing tuna, five containing sugar water solution) were placed out in linear fashion at 2-3 m intervals within each of the 20 plots. As with the pre-treatment ant survey, dispersal of the baited vials began at 9 a.m. C.S.T., and collection of the vials began at 10 a.m. C.S.T. Vials were capped upon collection and taken back to a laboratory setting, where species identification and estimation of the number of each species of ant per vial were determined. The experiment was continued in this manner each day for the first 7 d, with an additional survey conducted 2 wk later, and a final survey conducted 4 wk from the previous survey. Thus, ultimately, data sets consisting of ant species richness and abundance, both before treatment and at regular intervals after treatment, were produced for the treated plots and the control plots. These data were then used to determine which species of ants were susceptible to Advion™, as well as the longevity of control of these species afforded by label-rate broadcast treatment with 0.045% Advion™.

Additionally, soil temperature and precipitation were measured during this experiment. Soil temperature was measured with a Sergeant-Welch 12.7 cm soil thermometer (Sergeant-Welch, Buffalo Grove, IL) each morning within each of the plots for the first 7 d of the experiment, and then again on the two subsequent mornings when additional ant surveys were conducted. Precipitation was measured with a Garden

Treasures® 15.2 cm capacity rain gauge (Lowe's Companies Inc., Wilkesboro, NC) for each 24 h period for the first 7 d of the experiment. No 24 h precipitation readings were taken after the first 7 d.

Statistics. At the conclusion of this experiment, SPSS® software (SPSS 2001) was used to conduct statistical analysis of the data. First, ANOVA was conducted on the data sets resulting from the surveys of ant species richness and abundance before and after treatment, in both the treated plots and the control plots. Then, the LSD post hoc test was conducted on those same data sets to determine significant differences between the treatment group and the control group. All tests of significance were evaluated at $P = 0.05$.

Results

The mean morning soil temperature and the daily precipitation are shown in Table 28. The mean morning soil temperature was always 26.0° C or greater, and no precipitation occurred within 3 d of when the broadcast treatments were conducted. A comparison of the mean number of RIFA, *M. pharaonis*, *P. barbatus*, and *D. pyramicus* collected per plot in both the treatment group and the control group prior to treatment, and in subsequent days following treatment, is shown in Table 29 and Figure 17 ($F=6.48$; $df=79$; $P<0.05$). For the RIFA, when making comparisons within the treatment group, the mean number of ants found per plot 1 d prior to treatment was significantly greater ($P<0.05$) than the mean number of ants found on any given day following treatment.

Table 28. Mean morning soil temperature and daily precipitation for the experiment that evaluated the effectiveness of label-rate broadcast treatment with 0.045% Advion™ at controlling multiple ant species.

Day	Mean morning soil temperature ± SD (ºC)	Daily precipitation (cm)
0	27.6±1.0	0.0
1	28.1±0.7	0.0
2	27.9±1.0	0.0
3	27.5±0.7	0.0
4	26.0±0.5	3.3
5	26.6±0.4	0.3
6	26.8±0.5	1.0
7	27.1±0.5	0.3
21	27.9±0.8	—[a]
49	28.4±0.8	—[a]

[a] A precipitation reading was not taken on this day.

Table 29. Comparison of the mean number of *Solenopsis invicta*, *Monomorium pharaonis*, *Pogonomyrmex barbatus*, and *Dorymyrmex pyramicus* collected per plot in both the treatment group and the control group 1 d prior to treatment, and in subsequent days following treatment. The plots in the treatment group were subjected to label-rate broadcast treatment with 0.045% Advion™, and the plots in the control group were left untreated.

Day	Mean number of ants collected per plot, per species in treated plots and untreated plots pre-treatment and post-treatment[ab] (mean± SD)							
	S. invicta-plots untreated	*S. invicta*-plots treated w/0.045% Advion™	*M. pharaonis*-plots untreated	*M. pharaonis*-plots treated w/0.045% Advion™	*P. barbatus*-plots untreated	*P. barbatus*-plots treated w/ 0.045% Advion™	*D. pyramicus*-plots untreated	*D. pyramicus*-plots treated w/0.045% Advion™
pre-treat	93.0±15.9abA	105.5±15.4aB	102.0±10.3adAB	108.0±16.5aB	13.5±8.2aC	14.5±4.4aC	32.5±13.2abD	43.0±13.0aE
1	88.0±12.3aA	5.0±8.2bBE	105.0±16.5aC	9.0±8.7bBE	13.0±6.8aDE	0.0±0.0bB	34.0±14.1abF	2.0±3.5bB
2	96.0±16.3abA	0.0±0.0bB	115.5±15.7bC	8.5±6.3bBD	14.5±4.4aD	0.0±0.0bB	37.0±10.6aE	0.0±0.0bB
3	100.0±14.1bA	0.0±0.0bB	104.5±16.7aA	9.0±7.2bBC	13.0±4.8aC	0.0±0.0bB	31.5±15.6abD	0.0±0.0bB
4	113.5±16.5cA	0.0±0.0bB	103.0±17.7aC	9.5±7.9bD	14.0±3.9aD	0.0±0.0bB	29.0±12.0abE	0.0±0.0bB
5	138.5±12.5deA	1.5±4.7bB	92.0±9.5cC	14.5±10.7bD	13.0±5.4aD	0.0±0.0bB	30.0±10.0abE	1.5±4.7bB
6	134.0±12.0dA	2.5±5.4bB	90.5±13.4cfC	30.5±13.0cD	12.5±7.6aE	0.0±0.0bB	32.5±7.9abD	1.0±3.2bB
7	146.0±15.8efA	3.0±6.8bB	93.5±10.3cdC	30.0±12.7cD	12.5±2.6aE	0.0±0.0bB	31.5±10.3abD	5.0±8.5bBE
21	150.0±14.1fA	29.5±12.1cB	78.0±12.5eC	53.5±17.3dD	13.5±10.3aE	3.0±4.2bF	25.0±12.4bB	14.5±11.9cE
49	143.0±14.2defA	36.5±14.4cB	82.0±14.2efC	136.0±15.6eA	13.5±7.5aD	1.5±2.4bE	28.0±9.1abB	29.5±11.9dB

[a] Means within a column with different lowercase letters are significantly different at P<0.05. Means were separated using the LSD test.

[b] Means within a row with different uppercase letters are significantly different at P<0.05. Means were separated using the LSD test.

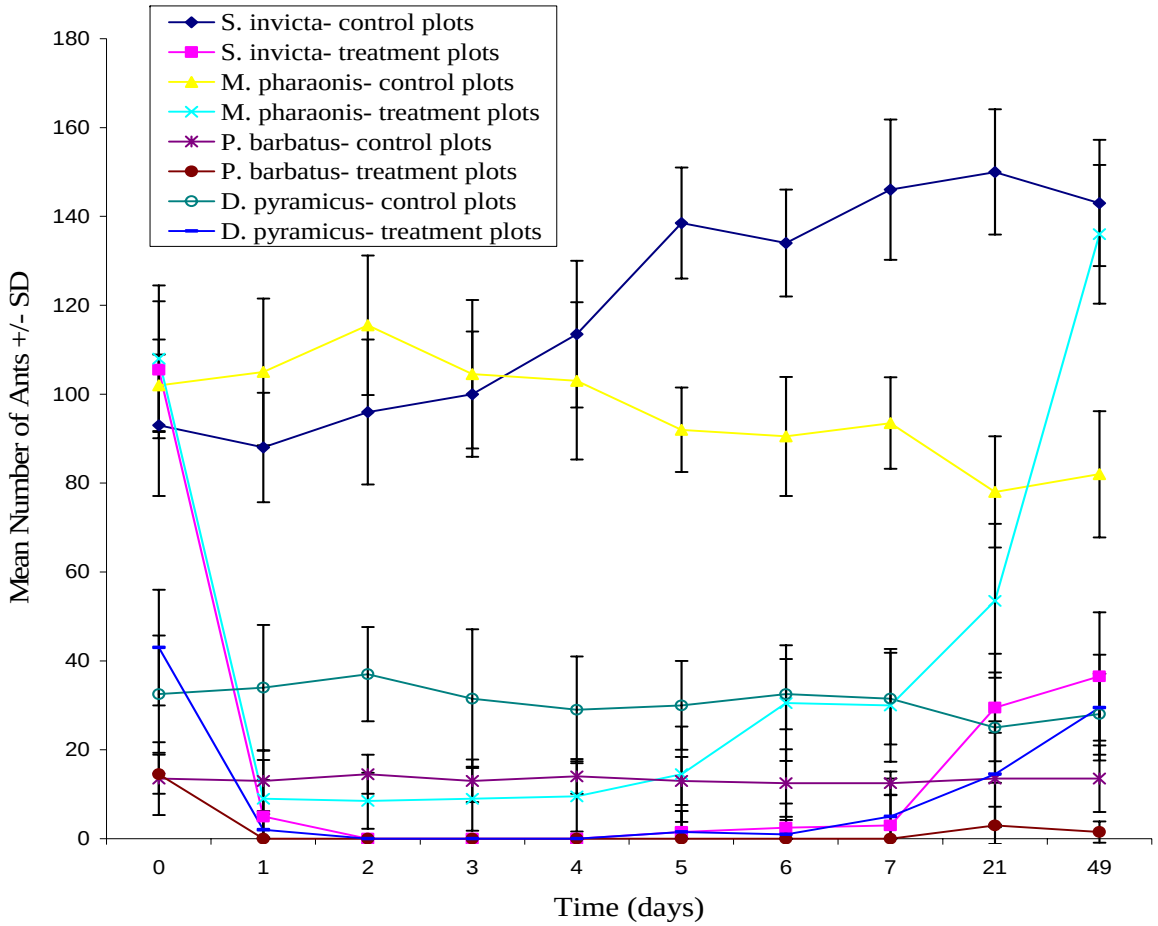

Figure 17. A comparison of the mean number of *Solenopsis invicta*, *Monomorium pharaonis*, *Pogonomyrmex barbatus*, and *Dorymyrmex pyramicus* collected per plot in both the treatment group and the control group 1 d prior to treatment, and in subsequent days following treatment. The plots in the treatment group were subjected to label-rate broadcast treatment with 0.045% Advion™, and the plots in the control group were left untreated.

There was no significant difference ($P>0.05$) in the mean number of ants found per plot for days 1-7 following treatment, and the mean number of ants found per plot on each of those days was significantly less ($P<0.05$) than the mean number found on any other day of the experiment. There was no significant difference ($P>0.05$) between the mean number of ants found per plot 21 and 49 days after treatment, and though the mean number of ants found per plot on both of those days was significantly greater ($P<0.05$) than the mean number found during the first 7 d following treatment, it was still significantly less ($P<0.05$) than the mean number of ants found 1 d prior to treatment. When comparing the treatment group to the control group, though the mean number of ants found per plot was significantly greater ($P<0.05$) in the treatment group than in the control group 1 d prior to treatment, the mean number was significantly less ($P<0.05$) in the treatment group than the control group for every day of the experiment following treatment.

In terms of *M. pharaonis*, when making comparisons within the treatment group, the mean number of ants found per plot 1 d prior to treatment was significantly greater ($P<0.05$) than the mean number of ants found per plot on days 1-7 and 21 following treatment. There was no significant difference ($P>0.05$) in the mean number of ants found per plot for days 1-5 following treatment, and the mean number of ants found per plot on each of these days was significantly less ($P<0.05$) than the mean number found on any other day of the experiment. Though the mean number of ants found per plot on days 6, 7, and 21 following treatment was significantly less ($P<0.05$) than that found 1 d prior to treatment, the numbers had increased such that the mean number of ants found

on days 6, 7 and 21 following treatment was significantly greater ($P<0.05$) than the mean number found on days 1-5 following treatment. By day 49, the mean number of ants found per plot had actually increased to a level that was significantly greater ($P<0.05$) than the mean number of ants found 1 d prior to treatment. When comparing the treatment group to the control group, though there was no significant difference ($P>0.05$) between the two groups in terms of the mean number of ants found per plot 1 d prior to treatment, the mean number of ants found per plot was significantly greater ($P<0.05$) in the control group than in the treatment group for days 1-7 and 21 following treatment. On day 49 following treatment, however, the mean number of ants found per plot in the treatment group was actually significantly greater ($P<0.05$) than the mean number found in the control group.

For *P. barbatus*, when making comparisons within the treatment group, the mean number of ants found per plot 1 d prior to treatment was significantly greater ($P<0.05$) than the mean number of ants found on any given day following treatment. No ants were found on days 1-7 following treatment, and there was no significant difference ($P>0.05$) in the mean number of ants found per plot among all days following treatment. When comparing the treatment group to the control group, though there was no significant difference ($P>0.05$) between the two groups in terms of the mean number of ants found per plot 1 d prior to treatment, the mean number of ants found per plot was significantly greater ($P<0.05$) in the control group than in the treatment group for every day of the experiment following treatment.

With relation to *D. pyramicus*, when making comparisons within the treatment group, the mean number of ants found per plot 1 d prior to treatment was significantly greater ($P<0.05$) than the mean number of ants found on any given day following treatment. There was no significant difference ($P>0.05$) in the mean number of ants found per plot for days 1-7 following treatment, and the mean number of ants found per plot on each of these days was significantly less ($P<0.05$) than the mean number found on any other day of the experiment. On day 21 following treatment, the mean number of ants found per plot was significantly greater ($P<0.05$) than the mean number found on days 1-7 following treatment, and the mean number of ants found per plot on day 49 was significantly greater ($P<0.05$) than the mean number found on day 21, though the mean number of ants found on both of those days was still significantly less ($P<0.05$) than the mean number found 1 d prior to treatment. When comparing the treatment group to the control group, though the mean number of ants found per plot was significantly greater ($P<0.05$) in the treatment group than in the control group 1 d prior to treatment, the mean number was significantly less ($P<0.05$) in the treatment group than the control group for days 1-7 and 21 following treatment. There was no significant difference ($P>0.05$) between the two groups on day 49 following treatment.

Discussion and Conclusions

It is well known that corncob grit-based RIFA baits, which are very effective due to the fact that they take advantage of the complex, trophallaxis-driven, social feeding behavior exhibited by many ant species, are often attractive to and effective against

numerous ant species in addition to the target species (Drees et al. 1996). While research has been conducted on numerous RIFA baits in an attempt to determine which ant species are affected by those baits, such research has not been conducted on the new RIFA bait Advion™ (Apperson et al. 1984; Drees and Gold 2003). Thus this research was conducted in an attempt to determine the effectiveness of label-rate broadcast treatment with Advion™ at controlling multiple ant species, with the research ultimately providing data on the effectiveness of Advion™ at controlling four different species of ants: the RIFA, *M. pharaonis*, *P. barbatus*, and *D. pyramicus*.

For this experiment, as with any experimentation conducted on RIFA in the field, it was important to ensure that the bait was broadcast when the soil temperature was within the optimal foraging range of the ants (22-36° C) and when a rainfall event was not expected for one or more days following treatment (Drees et al. 1996; Vinson 1997). As can be seen in Table 28, the mean soil temperature was always between 26° C and 29° C, and there was no rainfall event within 3 d of when the broadcast treatments were conducted. These data indicated that the bait was available during peak foraging periods, and that the bait was not adversely affected by a rainfall event, thus validating the timing of treatment for this experiment.

When comparing the treatment and the control group for each of the four species of ants included in this study, the data in Table 29 revealed that in terms of the mean number of ants found per plot 1 d prior to treatment there was either no significant difference ($P>0.05$) between the treated plots and the untreated control plots (*M. pharaonis* and *P. barbatus*), or there was a significantly greater ($P<0.05$) mean number

of ants in the treated plots than in the untreated control plots (the RIFA and *D. pyramicus*). However, for the first 7 d immediately following treatment, a significantly greater ($P<0.05$) mean number of ants was found in the untreated control plots than the treated plots for all four species. When making comparisons within the treatment group, for all four species a significantly greater ($P<0.05$) mean number of ants was found 1 d prior to treatment than on any given day for the first 7 d of the experiment following treatment. Additionally, the decrease in the mean number of ants found per plot from 1 d prior to treatment to 1 d after treatment was ~95% for the RIFA, ~92% for *M. pharaonis*, 100% for *P. barbatus*, and ~95% for *D. pyramicus*. Similarly, compared to the mean number of ants found per plot 1 d prior to treatment, the mean number 7 d after treatment was ~97% less for the RIFA, ~72% less for *M. pharaonis*, 100% less for *P. barbatus*, and ~88% less for *D. pyramicus*. These data clearly indicated that for each of the four species, broadcast treatment with Advion™ drastically reduced the mean number of ants found per plot after only 1 d, and for all four species that number remained only a small fraction of their respective pre-treatment number for at least 7 d following treatment.

With relation to the treatment group, for the RIFA and *P. barbatus* the mean number of ants found per plot following treatment remained significantly lower ($P<0.05$) than both the mean number of ants found prior to treatment and the mean number of ants found in the control group, for the duration of the experiment. Compared to the pre-treatment numbers, the mean number of ants found per plot 7 wk following treatment was ~65% less for the RIFA, and ~90% less for *P. barbatus*. For *M. pharaonis* and *D.*

pyramicus, though the mean number of ants found per plot in the treatment group 3 wk after treatment was still significantly less ($P<0.05$) than both the pre-treatment number and the number in the corresponding control group, the mean number of ants found per plot had increased significantly since day 7 following treatment for both species. This trend of increasing numbers continued and, after 7 wk, the mean number of *M. pharaonis* found per plot in the treatment group was actually significantly greater ($P<0.05$) than both the pre-treatment number and the number in the control group. Similarly, the numbers of *D. pyramicus* continued to increase, and after 7 wk there was no significant difference ($P>0.05$) between the mean number of ants found per plot in the treatment group and the control group, though the mean number found in the treatment group was still significantly less ($P<0.05$) than the pre-treatment number. However, though the mean number of *D. pyramicus* found per plot in the treatment group after 7 wk was still significantly less ($P<0.05$) than the pre-treatment number, it should be noted that the pre-treatment number in the treatment group was significantly greater ($P<0.05$) than the pre-treatment number in the control group, and there was no significant difference ($P>0.05$) between the mean number of *D. pyramicus* found per plot in the treatment group after 7 wk and the number found in the control group on any day of the experiment, including pre-treatment. Collectively, all of the aforementioned data indicated that there was a difference among species in the duration of control afforded by broadcast treatment with Advion™, as control of the RIFA and *P. barbatus* was maintained for the 7 wk duration of the experiment, and control of *M. pharaonis* and *P. barbatus* was maintained for some period of time greater than 3 wk but less than 7 wk.

In conclusion, when compared to pre-treatment levels, label-rate broadcast treatment with Advion™ resulted in a 95-100% decrease in the mean number of RIFA, *M. pharaonis*, *P. barbatus*, and *D. pyramicus* found per plot after only 1 d. Furthermore, 7 d after treatment the mean number of ants found per plot for each of these four species was still ~72-100% less than its respective pre-treatment number. There was a difference in the duration of control afforded by the treatment, as evidenced by the fact that the populations of *M. pharaonis* and *D. pyramicus* recovered far more quickly than did the populations of the RIFA and *P. barbatus*. Ultimately, label-rate broadcast treatment with Advion™ resulted in control of the RIFA and *P. barbatus* for at least 7 wk, and it resulted in control of *M. pharaonis* and *P. barbatus* for some period of time between 3 and 7 wk.

In order to more thoroughly address the effectiveness of label-rate broadcast treatment with Advion™ at controlling multiple ant species, additional field experiments similar to this one should be conducted, ideally in multiple localities so as to include a greater variety of ant species. Additionally, such studies should be conducted at different times of the day and night. All data collection for this experiment was conducted during the morning hours, thus biasing the study toward ant species that forage during that time. This bias could be overcome by conducting similar studies at different times of the day and night, which would help to maximize the number of ant species evaluated. This study was also biased toward ant species that recruited to the two food sources, sugar water and tuna, which were utilized in the baited vials. In future studies, the utilization of a greater variety of food sources in the baited vials would

prevent such bias, and it would also help to maximize the number of ant species evaluated in the study. Furthermore, in future research, it would be ideal to conduct more than a single pre-treatment ant survey in order to have a more substantial population baseline with which to compare post-treatment ant populations.

CHAPTER VII

DISCUSSION AND CONCLUSIONS

Being new to the urban marketplace, little research on Advion™ has been conducted and published, thus leaving numerous gaps in knowledge concerning this product. This research attempted to address several of those gaps in knowledge, ultimately resulting in a considerable quantity of data related to the following five specific objectives: 1) to determine the most effective chemical form and concentrations of indoxacarb, as well as the most appropriate grit size, for use in Advion™ in an attempt to ensure that the bait is maximally effective against the RIFA in terms of overall mortality and speed of mortality; 2) to determine the most effective concentration and quantity of Advion™, as well as the most effective placement of the bait, for obtaining maximum control of RIFA colonies via individual mound treatments with Advion™; 3) to compare the effectiveness of label-rate broadcast treatment with Advion™ to the effectiveness of both label-rate broadcast treatment with the RIFA bait Amdro® and pre-baiting broadcast treatment with Advion™; 4) to demonstrate that the indoxacarb within Advion™ is passed via trophallaxis, and to determine whether the RIFA worker caste is capable of metabolizing indoxacarb into the toxic JT333; and 5) to address the existing gap in knowledge concerning the effects of Advion™ on multiple ant species by evaluating the effectiveness of label-rate broadcast treatment with Advion™ at controlling *M. pharaonis*, *P. barbatus*, and *D. pyramicus*, in addition to the RIFA.

The results of the experiments conducted to achieve the first objective indicated that Advion™ containing indoxacarb was more effective at controlling the RIFA than Advion™ containing JT333, 0.045% was the most appropriate concentration of indoxacarb for use in Advion™ out of the three most effective concentrations (0.10%, 0.06%, and 0.045%) that were tested, and standard sized grit was more appropriate than small sized grit for use in Advion™. It should also be noted that for all three experiments the RIFA in the control groups exhibited significantly greater ($P<0.05$) overall feeding activity than did the RIFA that were treated with any concentration of Advion™ above 0.025%. Beginning with 0.025% Advion™, the lower the concentration, the more similar the feeding activity was to that exhibited in the control groups. This appeared to indicate that the RIFA were more sensitive to indoxacarb at levels above 0.025%.

Future experimentation should examine different concentrations of Advion™ other than the five that were evaluated in this study, focusing on those concentrations between 0.015% and 0.045%. Additionally, different grit sizes should be evaluated, primarily those varying in size between the standard sized grit and the small sized grit used in this study. Furthermore, Advion™ containing concentrations of JT333 that are less than the 0.045% concentration used in this study should be utilized and evaluated to determine if a lower concentration of the metabolite would be more effective.

Experiments conducted to achieve the second objective indicated that 10 g of 0.045% Advion™ was the most appropriate concentration and quantity of the eight concentrations and quantities (5-20 g of 0.045% Advion™, 15-20 g of 0.016%

Advion™, and 15-20 g of 0.008% Advion™) that proved to be effective as individual RIFA mound treatments. Furthermore, the data indicated that there was no significant difference ($P>0.05$) in colony mortality when 10 g of 0.045% Advion™ was placed around a RIFA mound in a circle with a radius of 0.5 m, 1 m, 2 m, or 3 m. Resultantly, based upon the data produced from this research, it was determined that 10 g (2 Tbsp) of 0.045% Advion™ placed around a RIFA mound in a circle with a radius of 0.5-3.0 m was the most effective manner in which to treat an individual RIFA mound with Advion™.

Future research should include multiple concentrations of Advion™ other than those tested in this research, ideally those between 0.016% and 0.045%. It would also be ideal to take daily readings for the duration of the experiment, unlike in this research in which daily readings were not taken during the second week, to produce more precise data on colony mortality for those treatments that resulted in significant colony mortality during the second week. Additionally, the length of future experiments should be greater than the 14 d duration of the experiments in this study in order to determine longevity of RIFA control in areas subjected to individual mound treatments. It would then be possible to compare the data derived from such individual mound treatment experiments to data derived from experiments conducted on broadcast treatments with Advion™ in order to evaluate the differences in speed of colony mortality and longevity of control resulting from these two different treatment types.

Results of the experiments conducted to achieve the third objective revealed that both pre-baiting broadcast treatment and label-rate broadcast treatment with 0.045%

Advion™ ultimately resulted in the same high level of RIFA colony mortality (98-99%), which was significantly greater ($P<0.05$) than that resulting from the other treatments. Further, there was no significant difference ($P>0.05$) in the mean number of new RIFA mounds observed per plot over the course of this experiment for the two aforementioned treatment groups. However, though pre-baiting broadcast treatment and broadcast treatment with 0.045% Advion™ ultimately achieved the same level and duration of RIFA control, broadcast treatment with 0.045% Advion™ was determined to be the most effective treatment overall due to the fact that it resulted in an LT_{90} of only 6.2 d, which was approximately half the LT_{90} resulting from pre-baiting broadcast treatment.

In order to further validate these results, this experiment should be repeated. In future research, it would be ideal to use various quantities of 0.0% Advion™ in the pre-baiting experiment to determine whether pre-baiting is, in fact, less effective due to a dilution effect. Additionally, the length of time it takes for the RIFA to re-colonize an area that has been subjected to broadcast treatment with a bait is highly variable, and largely dependent upon the number of RIFA colonies located on the land adjacent or in close proximity to the treatment area (Drees et al. 1996). Although for this experiment there was only one new RIFA colony observed after 49 d on the plots that were treated in some manner (i.e. pre-baiting broadcast treatment or label-rate broadcast treatment) with 0.045% Advion™, numerous studies of varied duration in various environments need to be conducted to definitively determine the longevity of control afforded by label-rate broadcast treatment with 0.045% Advion™.

Experimentation related to the fourth objective indicated that neither the Calco blue dye nor the fluorescent spray paint used in the experiment had any adverse effect on RIFA workers. Having established that, the results of the experiment revealed that indoxacarb delivered via Advion™ was passed among the RIFA via the process of trophallaxis, as demonstrated by both RIFA workers and RIFA larvae. And, finally, the data from this research clearly indicated that RIFA workers were capable of metabolizing indoxacarb into JT333.

As with all research, duplication is necessary to further validate these conclusions, and future research should ideally include more than three replicates. Future research should also attempt to determine the number of RIFA workers and larvae containing Calco blue dye by crushing all workers and microscopically examining all larvae in selected replicates after various amounts of time, such as after 48 h and 72 h. This would provide a set of data detailing the flow of indoxacarb through the workers and larvae within a RIFA colony over a clearly defined period of time. Additionally, for the replicates that were observed each day for 30 d, dead RIFA workers should ideally be removed each day and crushed to determine the presence or absence of Calco blue dye within the dead workers. This would provide further statistical evidence in support of the correlation between the presence of Calco blue dye and the presence of indoxacarb within RIFA workers.

Finally, the results of the experiment conducted to achieve the fifth objective revealed that when compared to pre-treatment levels, label-rate broadcast treatment with Advion™ resulted in a 95-100% decrease in the mean number of RIFA, *M. pharaonis*,

P. barbatus, and *D. pyramicus* found per plot after only 1 day. Furthermore, 7 d after treatment the mean number of ants found per plot for each of these four species was still ~72-100% less than its respective pre-treatment number. There was a difference in the duration of control afforded by the treatment, as evidenced by the fact that the populations of *M. pharaonis* and *D. pyramicus* recovered far more quickly than did the populations of the RIFA and *P. barbatus*. Ultimately, label-rate broadcast treatment with Advion™ resulted in control of the RIFA and *P. barbatus* for at least 7 wk, and it resulted in control of *M. pharaonis* and *P. barbatus* for some period of time between 3 and 7 wk.

In order to more thoroughly address the effectiveness of label-rate broadcast treatment with Advion™ at controlling multiple ant species, additional field experiments similar to this one should be conducted, ideally in multiple localities so as to include a greater variety of ant species. Additionally, such studies should be conducted at different times of the day and night. All data collection for this experiment was conducted during the morning hours, thus biasing the study toward ant species that forage during that time. This bias could be overcome by conducting similar studies at different times of the day and night, which would help to maximize the number of ant species evaluated. This study was also biased toward ant species that recruited to the two food sources, sugar water and tuna, which were utilized in the baited vials. In future studies, the utilization of a greater variety of food sources in the baited vials would prevent such bias, and it would also help to maximize the number of ant species evaluated in the study. Furthermore, in future research, it would be ideal to conduct

more than a single pre-treatment ant survey in order to have a more substantial population baseline with which to compare post-treatment ant populations.

In conclusion, this research produced a considerable amount of conclusive data related to the utilization and evaluation of the new RIFA bait Advion™. However, much research still needs to be conducted. Though these data add considerably to the sparse collection of published data related to Advion™ that is currently available, there are still numerous areas that require either additional or initial investigation. Additional research on the role of each caste with relation to metabolism of indoxacarb within a RIFA colony, the stepwise trophallactic flow of indoxacarb through a RIFA colony, and the effects of Advion™ on ant species (both pest species and beneficial species) other than the RIFA, as well as initial research on the photo stability and thermal stability of Advion™, all still need to be conducted due to existing gaps in knowledge. Ideally, future research will address specific topics such as these, and others, thereby continuing to fill in the existing gaps in knowledge related to Advion™.

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