Speed of Efficacy and Delayed Toxicity Characteristics of Fast-Acting Fire Ant (Hymenoptera: Formicidae) Baits

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ABSTRACT Efficacy and speed of action of fire ant (Hymenoptera: Formicidae) baits that claim fast control of colonies were compared with a standard bait. More than 85% of red imported fire ant, *Solenopsis invicta* Buren, laboratory colonies provided bait containing the active ingredient indoxacarb died within 3 d, and all colonies were dead in 6 d. Standard bait containing hydramethylnon resulted in death of 60% of the colonies in 9 d. Bait containing spinosad did not cause colony death. Under field conditions, one-half of the areas treated with the indoxacarb bait did not have any active fire ant nests within 3 d, whereas 11 d was needed to reach the same level of control with the hydramethylnon bait. Spinosad had a maximum of 17% of the treated areas without nests after 3 d. The delay in death of *S. invicta* adults treated in the laboratory with the indoxacarb and spinosad baits was shorter than the standard hydramethylnon bait, which had mortality similar to the traditional delayed toxicity criterion of <15% mortality after 24 h and >89% mortality over the test period. Indoxacarb caused mortality of 57% at 24 h and 100% at 48 h; however, visual symptoms of toxicity were not readily observed for at least 8 h before the abrupt increase in death. Spinosad caused 96% mortality by 24 h, and initial mortality became apparent at 4 h. Time required for death of 15% of a treated population (LT_{15}) of spinosad, indoxacarb, and hydramethylnon was 3, 9, and 16 h, respectively. Delayed toxicity characteristics of the fast-acting indoxacarb bait may be useful for the development of other fast-acting ant baits.

KEY WORDS *Solenopsis invicta*, red imported fire ant, bait, delayed toxicity, chemical control

The red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is an invasive ant that has become a dominant arthropod pest in the southern United States since its accidental introduction in the 1930s (Williams et al. 2001). It causes greater than $6.5 billion in estimated losses to the agricultural and urban sectors each year (Pereira et al. 2002). There is potential for further range expansion of this pest both in the United States and globally (Morrison et al. 2004). This potential has been validated by the discovery of infestations in California in 1997 (Jetter et al. 2002); Australia in 2001 (Henshaw et al. 2005); Taiwan in 2004; and in Hong Kong, mainland China, and Mexico in 2005. The destructive significance of *S. invicta* and its geographic expansion necessitate the continual improvement in control methods.

The use of insecticidal baits can be a very effective method of fire ant control, because it allows for the distribution of toxicant throughout a colony (Williams et al. 2001). Depending on the active ingredient, fire ant baits have typically required weeks to months to significantly reduce ant populations (Drees et al. 2000). This time delay represents a hazard that may be intolerable in sensitive environments such as daycare and healthcare facilities, where there exists the risk of severe stings to young children or the infirm (deShazo et al. 2004). For more immediate population reductions, treatment of individual nests with faster acting contact insecticides has been used. This method of control generally is less efficient than baiting because nests must be located and be accessible to allow for thorough insecticide contact with the colony (Barr et al. 1999). Recently, two fire ant baits have been marketed with claims of controlling fire ant colonies as fast as 1–3 d. The objectives of this study were to 1) determine the time needed to obtain colony mortality of *S. invicta* in the laboratory and field with fire ant baits that claimed fast efficacy and 2) examine delayed toxicity characteristics of these baits relative to their speed of efficacy against colonies and individual ants.

Materials and Methods

Speed of Efficacy on Laboratory Colonies. To determine the time required to reduce the brood and adults in colonies and kill queens, colonies of *S. invicta* that were reared from newly mated queens (Banks et al. 1981) were provided access to the fire ant baits

containing the active ingredients (AIs) spinosad (0.015% [AI], Ortho Fire Ant Killer Bait Granules, The Ortho Group, Columbus, OH), indoxacarb (0.045% [AI], registered in 2004 as Advion, E. I. du Pont Nemours, Wilmington, DE), hydramethylnon (0.73% [AI], Siege Pro, BASF, Research Triangle Park, NC), and a control of 30% by weight once-refined soybean oil (Vander Meer et al. 1982) absorbed into pregel, defatted, corn grit (Illinois Cereal Mills, Paris, IL). Soybean oil and corn grit are commonly used in commercial fire ant baits as a food bait and carrier, respectively. Approximately 59 ml of bait was provided to each colony, which was a recommended label rate (4 tablespoons) for the treatment of individual nests. To eliminate possible confounding effects of competing food sources on bait foraging, baits were the only food available to the colonies for 2 d before the regular laboratory diet of crickets and 10% (wt:vol) sugar–water solution was added. Baits remained with the colonies, without replenishment, for the duration of the study. Food was not provided to colonies for 0, 3, or 5 d before the introduction of baits to compare the effects of starvation on colony reduction among baits. Water was available throughout the study, including the starvation period. Each combination of the four bait treatments and the three starvation periods was replicated on five colonies, resulting in 60 experimental units.

Brood volume and adult numbers per colony were monitored daily for 7 d. The number of adults and brood volume were estimated through visual comparisons of photos with known colony sizes (Banks and Lofgren 1991). Colonies initially contained 37,063 ± 664 (mean ± SE) adults, 39.5 ± 1.1 ml of brood (eggs, larvae, and pupae), and one queen. Daily percentage of reductions in brood and adults relative to initial populations were averaged over days 1–7 and compared among baits and starvation periods with two-way analyses of variance (ANOVAs). Means were separated with Tukey's honestly significant difference (HSD) tests (PROC GLM, SAS Institute 2002). The logarithmic transformation $[\log_{10}(x + 100)]$ was used on brood and adult data to reduce heterogeneity among variances. Queen survivorship was monitored daily for 10 d, and then at 14, 18, or 21 d, and 38 d after the introduction of baits. The number of days for queen death to occur for the first 10 d was compared among each bait and starvation level by two-way ANOVA (PROC GLM, SAS Institute 2002). Time of death was compared for only the first 10 d because 27 of the 29 dead queens died within this time frame.

Speed of Efficacy on Field Colonies. The study site was a parking lot on the University of Florida campus in Gainesville, FL (Alachua Co.). The site contained islands of grass and small trees (30.4 ± 7.5 m^2, mean ± SD). Islands were separated by a minimum of 7.3 m of asphalt pavement. One island was adjacent to a concrete sidewalk. One day before treatment, each island was surveyed for fire ant nests as indicated by the presence of at least 100 fire ants in mounds of excavated soil. The size of fire ant colonies in the nests was estimated using the population index ratings (Lofgren

and Williams 1982), where nests are opened with a shovel and the number of adult ants and the presence of worker caste brood are assigned to colony size categories. Based on the number and size of colonies per island, a randomized complete block design was used to assign a single treatment to each island. In total, 24 islands were used for four treatments with six replicated blocks. Treatments consisted of applying fire ant bait to each nest on an island following label directions. In general, 59 or 89 ml (1/4 or 3/8 cup) of bait was distributed around the base of each nest. Fire ant baits used in the preceding laboratory study were applied on 15 April 2004. To limit the possibility of fire ants on the control islands feeding on other bait treatments, bait without toxicant, identical to that used for the controls in the preceding study, was applied to nests on the control islands. Air temperatures ranged from 22.8 to 26.1°C during bait application, and fire ants were observed foraging during and after application.

The number of active nests on each island was determined on the day baits were applied and on 1–8, 11–14, and 21 d after bait application. Nests were designated as active when at least 20 adult fire ants exited mounds of excavated soil probed with a metal rod (4.8 or 6.5 mm in diameter). Treatment evaluations were based on the percentage of reduction in active fire ant nests relative to the initial number of treated active nests, and the percentage of the 13 d sampled that each island did not contain any active nests. Percentage of reductions in active nests were compared among bait treatments by a repeated measure ANOVA and Ryan–Einot–Gabriel–Welsch multiple range test (SAS Institute 2002). Percentages of fire ant nest-free islands were compared by ANOVA and Ryan–Einot–Gabriel–Welsch multiple range test (SAS Institute 2002). The arcsine transformation was performed on percentages to reduce heterogeneity before analyses. In addition, the average number of active nests per sampling date were compared among treatments by ANOVA and Ryan–Einot–Gabriel–Welsch multiple range test (SAS Institute 2002). A significance level of $\alpha = 0.05$ was used for all analyses.

Delayed Toxicity to Adult Workers. Laboratory studies were conducted to compare delays in the death of adult *S. invicta* exposed to baits by using procedures that were adapted from the limited feeding test of Stringer et al. (1964) and the primary screening described by Williams (1983). Tests were conducted in plastic, 30-ml (1-fl.-oz.) cups where a hole (≈3 mm in diameter) was punched into the bottom of each cup, and then the cup filled approximately one-third full with dental plaster (Castone, Dentsply Trubyte Division, York, PA) that was allowed to harden. During a test, a humid environment was maintained by capping the cups and setting them on a water-saturated foam rubber pad, which allowed water to be wicked into the plaster. Interior sides of the cups were lightly dusted with talcum powder to prevent ant escape during observations.

Major workers (head widths of 1.16 mm ± 0.09 [mean ± SD]) obtained from laboratory colonies that

had been starved for 5 d were used in the study. Head widths were determined from a subsample of ants ($n = 151$) with a wedge micrometer (Porter 1983) at the conclusion of the study. Twenty ants were placed into the cups and held overnight. The next day, any dead ants were replaced with ants from the starved colony. A vial cap (1 ml) filled with bait or a control of pregel defatted corn grit mixed with once-refined soybean oil [30% (wt:wt)] was placed into each cup containing the ants. After 24 h, the caps containing the baits were removed and the ants held without food for an additional 24 h to allow for any trophallactic exchange of toxicant. Next, a cotton applicator tip saturated with the soybean oil was added, in another vial cap, to each cup for the remainder of the study. Dead or moribund ants were counted and removed after 2, 4, 6, 8, 12, 20, 24, 28, and 36 h, and 2, 3, 6, 8, 10, 13, and 14 d after the treatments were provided to the ants. Ants were considered moribund if they could not stand after being touched with a probe and were lethargic. The study was conducted under ambient temperature and relative humidity, which averaged $26.7 \pm 0.4°C$ (SD) and $73.7 \pm 4.4\%$, respectively. Treatments consisted of the baits used in the previous studies (i.e., baits containing indoxacarb, hydramethylnon, spinosad, and only soybean oil [control]), and were replicated 10 times, with ants from a separate colony being used for each replicate. Mean percentage of cumulative mortalities and standard errors were reported among baits. Lethal times (LT_{15}, LT_{50}, and LT_{90}), which is the time required for 15, 50, and 90% of the ants to die, respectively, and 95% confidence limits (CL) among baits were estimated from regression analyses of angular transformed cumulative percentage of mortality ($\text{arcsine}\sqrt{Y}/100$, where Y is cumulative percentage of mortality) on logarithmically transformed time (Sokal and Rohlf 1969). Mortality was corrected with Abbott's formula (Abbott 1925). For some treatments, analyses were conducted over a shorter time, because all ants died before 14 d.

The preceding study was repeated to compare delays in toxicity when abnormal behavior was considered in addition to mortality. Abnormal behavior was defined as ants exhibiting tremors, or shaking while standing, or as ants lying in a curled position, but able to stand, or actively attempted to stand, after being probed. It was assumed that ants displaying these behaviors would be incapable of trophallaxis. Treatments consisted of indoxacarb, hydramethylnon, or control bait. Spinosad bait was excluded, because delays in mortality were minimal or not evident in the previous test, hence preventing observations of abnormal behavior. LT_{15}, LT_{50}, and LT_{90} values were calculated from regression analyses on cumulative percentages based on the criteria of 1) ants exhibiting abnormal behavior plus those ants that were moribund or dead or 2) ants that were moribund or dead. After 2, 4, 6, 8, 12, 20, and 24 h and 2, 3, 6, 8, 10, 13, and 14 d after ants were given access to the baits, abnormal ants and moribund or dead ants were counted, and moribund or dead ants removed. Data for the indoxacarb treatment analysis encompassed 6 d, when all ants

Table 1. Percentage of reductions in brood volume averaged over days 1 through 7 after bait treatments were provided to S. invicta laboratory colonies starved for 0, 3, and 5 d ($n = 5$)

Treatment	Starvation period (d)			Total
	0	3	5	
Indoxacarb	71.8	90.3	86.7	83.0a[a]
Hydramethylnon	17.1	31.5	54.4	34.3b
Spinosad	7.3	9.2	32.3	16.3c
Control	−1.4	0.0	0.0	−0.5d
Total	23.7a[b]	32.7ab	43.4b	

Analyses conducted on $\log_{10}(x + 100)$-transformed data; untransformed means are presented. Treatment by starvation interaction was not significant ($F = 1.57$; df = 6, 44; $P = 0.18$).
[a] Means within a column followed by the same letter are not significantly different by Tukey's HSD test ($\alpha = 0.05$).
[b] Means within a row followed by the same letter are not significantly different by Tukey's HSD test ($\alpha = 0.05$).

were dead. There were 10 replicates, with ants for each replicate obtained from a different colony. Lethal times and 95% CL among baits and criteria for death were estimated from regression analyses of angular, probit, or square root-transformed cumulative percentage of mortality on logarithmically transformed time (Sokal and Rohlf 1969).

Results and Discussion

Speed of Efficacy on Laboratory Colonies. Percentage of reductions in brood volume were significantly different among all bait treatments ($F = 66.43$; df = 3, 44; $P < 0.001$). The largest average reduction over the first 7 d after bait introductions was in the indoxacarb treatment followed by hydramethylnon and spinosad baits. Brood volume did not change in the controls (Table 1). Similarly, percentage of reductions in adult numbers were significantly greatest ($F = 43.54$; df = 3, 44; $P < 0.001$) with the indoxacarb bait. The hydramethylnon and spinosad baits exhibited significantly higher worker death than the control (Table 2). Regardless of treatment, colonies starved for 5 d had significantly greater percent reductions in brood than the nonstarved colonies ($F = 7.38$; df = 2, 44; $P <$

Table 2. Percentage of reductions in the number of adult workers averaged over days 1 through 7 after bait treatments were provided to S. invicta laboratory colonies starved for 0, 3, and 5 d ($n = 5$)

Treatment	Starvation period (d)			Total
	0	3	5	
Indoxacarb	74.1	95.1	94.7	88.0a[a]
Hydramethylnon	16.5	50.5	54.8	40.6b
Spinosad	2.6	41.3	29.7	24.5b
Control	−9.4	−12.4	−8.6	−10.2c
Total	20.9a[b]	43.6b	42.6b	

Analyses conducted on $\log_{10}(x + 100)$-transformed data; untransformed means are presented. Treatment by starvation interaction was not significant ($F = 0.98$; df = 6, 44; $P = 0.45$).
[a] Means within a column followed by the same letter are not significantly different by Tukey's HSD test ($\alpha = 0.05$).
[b] Means within a row followed by the same letter are not significantly different by Tukey's HSD test ($\alpha = 0.05$).
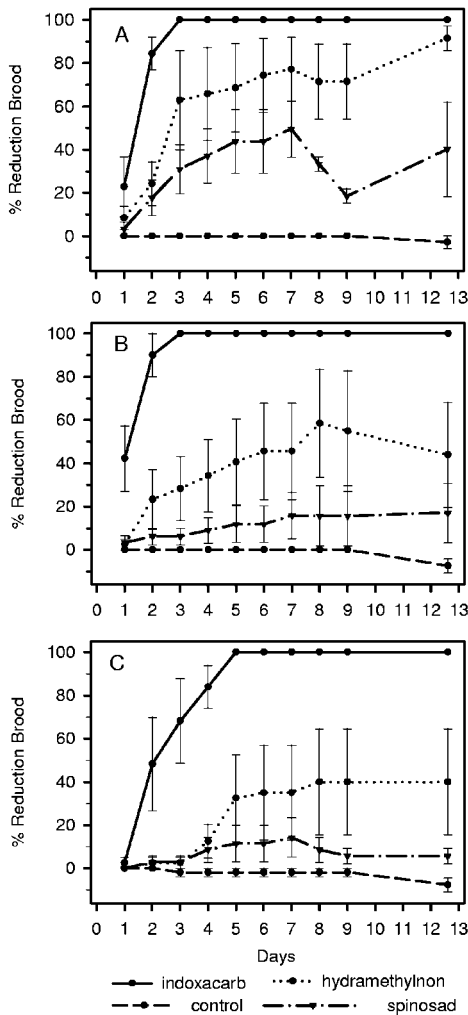
Fig. 1. Average ± SE ($n = 5$) percentage of reductions per colony in *S. invicta* brood among colonies given access to various fire ant baits after 5- (A), 3- (B), and 0 (C)-d starvation periods. Data points after day 12 represent data collected on either days 10, 14, or 15.
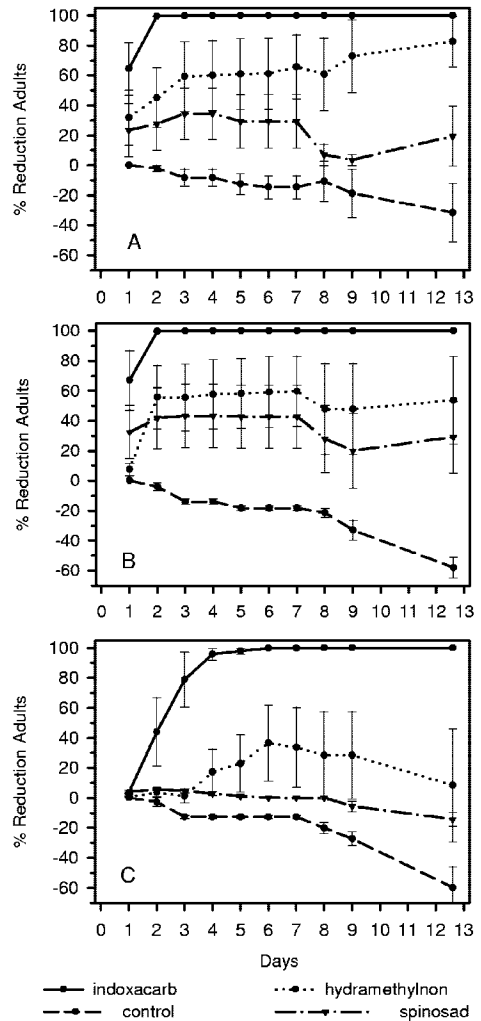

Fig. 2. Average ± SE ($n = 5$) percentage of reductions per colony in *S. invicta* adults among colonies given access to various fire ant baits after 5- (A), 3- (B), and 0 (C)-d starvation periods. Data points after day 12 represent data collected on either days 10, 14, or 15.

0.002) (Table 1), and adult populations were reduced significantly more for both starvation periods ($F = 5.31$; df = 2, 44; $P < 0.009$) (Table 2). Bait by starvation interaction was not significant for percentage of reductions in either brood or adults. Reductions in brood and adults were >95% 5 d after the indoxacarb bait introduction when colonies were not starved (Figs. 1C and 2C). Starved indoxacarb-baited colonies had >99% reductions in brood and adults within 3 d. Maximum mean reductions in the hydramethylnon-treated colonies occurred in the 5-d starvation period with reductions of 91% for brood and 83% for workers after 12 d. In the spinosad treatments, maximum mean reductions of 50 and 43% for brood and adults, respectively, were obtained within a week from 5-d- and 3-d-starved colonies, respectively (Figs. 1 and 2).

Queen mortality was highest in the indoxacarb treatment with all 15 queens dying within 6 d, followed by the hydramethylnon treatment with 13 of 15 queens dead within 38 d, and one queen dying in the spinosad treatment within 38 d. None of the queens in the control colonies died. The mean ± SE time to queen death over all starvation periods was 2.2 ± 0.4 d in the indoxacarb baited colonies. This time was significantly shorter than the 6.8 ± 0.6 d for the hydramethylnon-baited colonies ($F = 62.57$; df = 1, 17; $P < 0.001$). The effect of starvation periods on the time to queen death over the two baits was not significant. Bait by starvation interaction was not significant ($F = 3.49$; df = 2, 17; $P = 0.0538$), but queen mortality began to converge when colonies were not starved (Fig. 3). Because appreciable queen death occurred only in
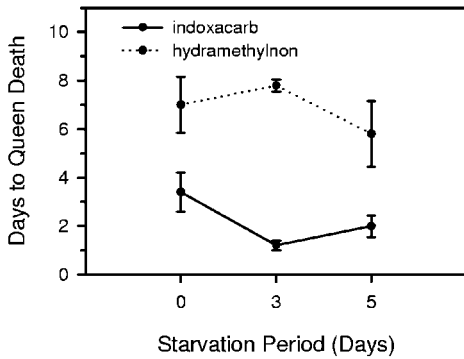
Fig. 3. Average ± SE number of days for queens to die in laboratory *S. invicta* colonies exposed to baits containing hydramethylnon or indoxacarb among 0-, 3-, and 5-d starvation periods. Data presented is for an observation period of 10 d. *n* = 5 for each combination of indoxacarb and starvation period. For hydramethylnon, *n* = 3, 4, and 5 for the 0-, 3-, and 5-d of starvation, respectively. Queen death did not occur in any of the control colonies, and one queen died in the spinosad treatment after 18 d. These data are not presented in the figure.

Fig. 4. (A) Percentage of reductions in active *S. invicta* nests per sampling date, relative to initial numbers of active nests, among bait treatments. (B) Percentage of parking lot islands without any active *S. invicta* nests per sampling date among bait treatments (*n* = 6 islands per treatment).

the indoxacarb- and hydramethylnon-baited colonies, these were the only treatments analyzed.

Using the criteria of ≥90% reduction in adult workers and a dead queen to designate a colony as being dead, 87% of the colonies baited with indoxacarb died by the third day, and all colonies were dead within 6 d. In contrast, a maximum of 60% of the colonies provided with the standard hydramethylnon bait died by the ninth day. This level of colony control with hydramethylnon is less than laboratory colony deaths reported by Williams et al. (1980) who used higher concentrations (2.5, 5.0, and 10.0% in soybean oil) of the active ingredient.

Speed of Efficacy on Field Colonies. Baits were applied to 2.9 ± 2.1 (mean ± SD, range 1–8) nests per island, and the number of nests baited among treatments was not significantly different ($F = 0.47$; df = 3, 15; $P = 0.71$). Based on an average population index rating of 16.2 ± 5.7, treated nests contained worker brood and had an estimated 1,000–10,000 adults. Percentage of reduction in the number of active nests was not significantly different ($F = 3.00$; df = 3, 20; $P = 0.0547$) among the treatments over the 21-d study period, because treatment reductions varied at different times. Reductions of >50% occurred in the indoxacarb treatment by the second day, and this level of control was sustained or increased for an additional 9 d. In contrast, only the hydramethylnon treatment exceeded 50% reduction after 10 d, and spinosad had fluctuating reductions between 10 and 41% (Fig. 4A). The more stringent standard of percentage of islands without any active fire ant nests was not significantly different ($F = 2.69$; df = 3, 15; $P = 0.0836$) among the treatments over the duration of the study (Table 3). However, on islands treated with the indoxacarb bait, active nests were not observed on 50% of the islands within 3 d after bait application, whereas islands in the
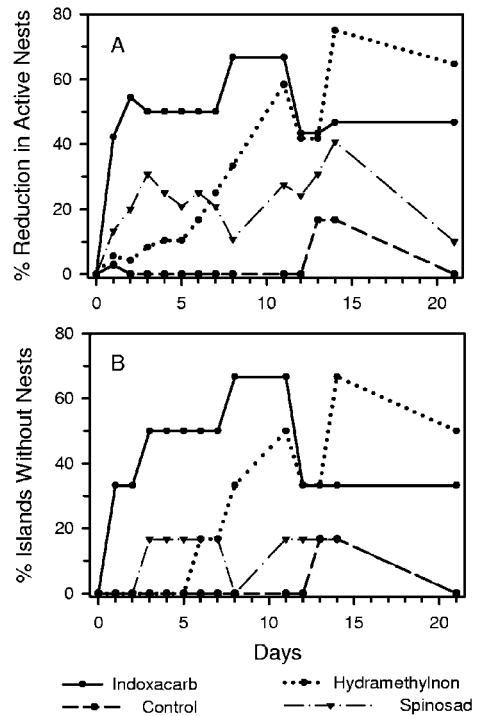
other treatments did not have any nests on 0–17% of the islands for the first 7 d. After 7 d, 33–67% of the islands treated with indoxacarb or hydramethylnon did not have active nests (Fig. 4B). The decline in the percentage of reductions of active nests and nest-free islands in the indoxacarb treatment after day 11 may be attributed to the immigration of colonies from neighboring islands or property beyond the study site. Colony movement among the parking lot islands is presumably well within capability of fire ants, which have been reported to forage at least 36 m (Martin et al. 1998). The newly detected colonies were estimated to have at least 100 ants; thus, it would be unlikely that they were produced by new, colony-founding queens. The spinosad treatment eliminated nests on one island in 3 d. Control islands had active nests on all islands throughout most of the study (Fig. 4). The absence of active nests in the indoxacarb treated islands within 1–3 d supports conclusions by Barr (2003, 2004) of broadcast applications of indoxacarb baits resulting in faster declines in active nests and foraging activity than the standard hydramethylnon bait. The spinosad treatment exhibited minimal control in our study; other reports have indicated both good and poor field efficacy with spinosad baits (Barr 1997, 2004; Hertl et al. 1999). Barr (1997) speculated that the dose range for effective spinosad distribution by trophallaxis was very narrow. Thus, variable colony sizes and foraging

Table 3.   Effect of bait treatments on fire ant nests located on parking lot islands over the duration of study

Bait	Initial no. active nests/island[a]	Avg. no. active nests/island/d after treatment[b]	Avg. % d islands were without active nests[c]	Avg. % reduction in active nests/island/d after treatment[d]
Hydramethylnon	2.7a	1.9a	23.1a	30.4a
Indoxacarb	2.7a	1.6a	44.9a	50.8a
Spinosad	2.8a	2.7a	11.5a	23.1a
Control	3.3a	4.7a	2.6a	2.8a

[a] Averages within a column followed by the same letter are not significantly different ($P > 0.05$) by ANOVA.

[b] Averages ($n = 6$) within a column followed by the same letter are not significantly different ($P > 0.05$) by ANOVA and Ryan–Einot–Gabriel–Welsch multiple range test.

[c] Averages ($n = 6$) within a column followed by the same letter are not significantly different ($P > 0.05$) by ANOVA and Ryan–Einot–Gabriel–Welsch multiple range test. Percentages based on 13 sample dates; analyses performed on arcsine-transformed data; untransformed means are presented.

[d] Averages ($n = 78$) within a column followed by the same letter are not significantly different ($P > 0.05$) by repeated measures ANOVA. Analyses performed on arcsine-transformed data; untransformed means are presented.

rates could result in the inconsistent ingestion of sufficient active ingredient resulting in varying efficacy.

The indoxacarb, hydramethylnon, and spinosad treatments had 65, 58, and 43% fewer active nests, respectively, than the control over the duration of the study. However, the average number of active nests per island per sample date did not differ statistically among treatments ($F = 2.52$; df = 3, 15; $P = 0.0976$; Table 3). This finding could be attributed partly to the phenomenon of treated colonies moving or splitting into several nests before they become inactive (Williams and Lofgren 1983, Barr and Drees 1995). In addition, active nests remained on some islands throughout the study. This finding suggested that perhaps colonies did not forage on the bait or that some colonies were not located and treated. A broadcast application of bait over an entire island may have assured bait accessibility to all colonies. However, at typical broadcast application rates of 1,680 g/ha (1.5 lb/acre), only 5 g of bait would be applied per 30-m^2 island. Distribution of such a small amount of bait is well below the label recommendations of 10–56.7 g per individual mound, or nest. This illustrates a dilemma of baiting fire ants in small areas, where broadcast rates may be insufficient, thus requiring the more inefficient method of locating and treating individual nests.

Delayed Toxicity to Adult Workers. Examination of the mean percentage of cumulative mortality profiles among treatments indicated that only the hydramethylnon bait approached the definition of Stringer et al. (1964) of delayed toxicity, where there is <15% mortality 24 h after initial bait access and >89% mortality by the end of the test (Fig. 5). The spinosad and the indoxacarb baits both exceeded the 15% mortality criterion by having >55% mortality at 24 h. However, the onset of mortality for the latter two baits differed. Death from spinosad occurred within 4 h and reached 99% by 48 h. Indoxacarb had no appreciable mortality (1.5%) for the first 8 h and attained 100% mortality by 48 h (Fig. 5B).

The LT$_{15}$ and the LT$_{50}$ values for the spinosad bait were below the respective 95% confidence intervals of the indoxacarb and hydramethylnon baits. Confidence intervals for the LT$_{15}$ values of indoxacarb and

hydramethylnon overlapped. For the LT$_{90}$ values, the confidence interval for hydramethylnon was greater than the overlapping intervals for spinosad and indoxacarb (Table 4). These results reflected the faster mortality with the spinosad and indoxacarb baits relative to hydramethylnon.

Comparison of cumulative mortality profiles based on either abnormal behavior plus moribund/dead or moribund/dead only depicted faster activity within a treatment when abnormal behavior was included (Fig. 6). Hydramethylnon LTs were significantly (95% confidence intervals did not overlap) lower when ab-
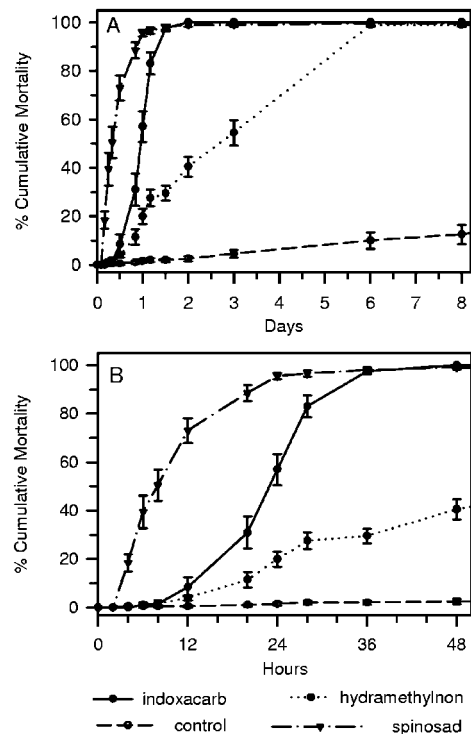

Fig. 5.   Average ± SE ($n = 10$) percentage of cumulative mortality of 20 adult, *S. invicta* workers given access to fire ant bait for 24 h. Mortality was recorded over a 14-d period. (A) Data for days 0–8. (B) Data for the first 48 h.

Table 4. Lethal times in days, 95% CL, regression equations, and coefficients of determination (R^2) for adult *S. invicta* workers provided fire ant baits containing hydramethylnon, indoxacarb, or spinosad

Bait (% AI)	LT_{15} (d) (95% CL)	LT_{50} (d) (95% CL)	LT_{90} (d) (95% CL)	Regression equation $y = a + bx^a$	R^2
Hydramethylnon (0.733)	0.65 (0.47–0.89)	1.83 (1.35–2.48)	6.34 (4.16–9.78)	$y^b = -0.622 + 0.858x$	0.88
Indoxacarb (0.045)	0.37 (0.26–0.52)	0.79 (0.56–1.12)	1.96 (1.40–2.80)	$y^c = -0.720 + 1.177x$	0.81
Spinosad (0.015)	0.12 (0.09–0.17)	0.34 (0.24–0.47)	1.12 (0.80–1.58)	$y^c = -0.028 + 0.894x$	0.82

Lethal times were estimated from regression of angular transformed ($\mathrm{arcsine}\sqrt{Y}$) mortality (mortality corrected by Abbott's formula) on hours logarithmically transformed. Backtransformed LTs and CLs are presented in days.

[a] x, $\log_{10}$(hour).
[b] Regression over hours 2–312; $n = 150$.
[c] Regression over hours 2–144; $n = 120$.

normal behavior was added to the moribund/dead, but not with indoxacarb (Table 5). When abnormal behavior was included with mortality, the initial visual symptoms of toxicity was 4 and 14 h earlier with indoxacarb and hydramethlynon, respectively. For LT_{15} values, the indoxacarb treatment was 7.4 h and hydramethylnon was 43 h earlier with the inclusion of abnormal behavior. The LT_{15} and LT_{50} CLs did not overlap for hydramethylnon based on moribund/dead data obtained from the two separate delayed toxicity tests (Tables 4 and 5). Variation in delayed toxicity
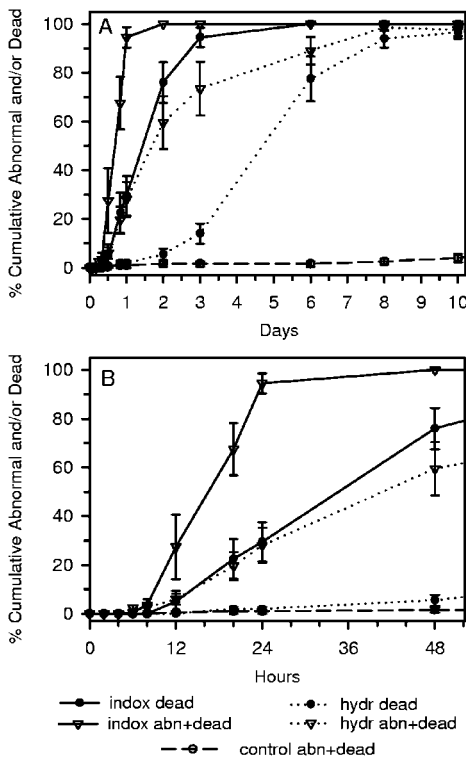

Fig. 6. Average ± SE ($n = 10$) cumulative percentages of 20 adult, *S. invicta* workers given access to fire ant bait for 24 h that died, and the percentages that exhibited abnormal behavior and/or were dead. Abnormal behavior and mortality were recorded over a 14-d period. (**A**) Data for days 0–10. (**B**) Data for the first 48 h. hydr, hydramethylnon; indox, indoxacarb; abn, abnormal behavior.

among separate tests has occurred with mirex and hydramethylnon baits (Banks et al. 1977, Vander Meer et al. 1982). The differences may be attributed to many unspecified factors among different studies (e.g., temperature, ant size, and nutritional needs) that could contribute to experimental variation.

As demonstrated herein, the criterion used to designate mortality can affect the determination of whether a toxicant is selected for further development as ant bait relative to delayed toxicity and lethal times. Onset and duration of symptoms of poisoning before death can be affected by factors such as mode of action and dose ingested. Reid and Klotz (1992) and Klotz and Reid (1993) reported that using death as a criterion resulted in LT_{50} values for black carpenter ants, *Camponotus pennsylvanicus* (De Geer), that were <1 d later for nerve poisons, to a few days later for respiratory inhibitors, compared with using moribundity (ataxia, excitation, lethargy, and paralysis). Note that their studies used a broader definition of moribundity than what was used in the current study where moribundity was equated to death, and abnormal behavior encompassed ataxia and excitation (tremors/shaking) and lethargy (lying, curled body but capable of standing when probed). However, in either case, symptoms expressed before death were assumed to impair an ant's normal behavior and thus interfere with the trophallactic distribution of toxicant.

Designations of delayed toxicity have been based on various methods and interpretations of data. Klotz and Reid (1993) used LT_{50}s and effective lethal time to 90% of the population based on direct (primary) and indirect (secondary) ingestion of toxicants by *C. pennsylvanicus*. Toxicants were designated as having a delayed action if 90% mortality was still obtained, albeit over longer periods, as toxicant concentrations decreased. In contrast, acute toxicants would cause lower percentages of mortality with lower concentrations, even after a long postexposure period (Su et al. 1987). Low concentrations of boric acid in sucrose solution were considered to cause delayed toxicity in the Florida carpenter ant *Camponotus floridanus* (Buckley), based on relatively stable LT_{50} values over 7 d (Klotz and Moss 1996). For *S. invicta*, low concentrations of boric acid in sucrose solution were considered to be nonrepellent and slow-acting based on LC_{50} values over 3–8 d (Klotz et al. 1997). For

Table 5. Lethal times, 95% CL, regression equations, and coefficients of determination (R^2) for adult *S. invicta* workers provided fire ant baits containing hydramethylnon or indoxacarb

Bait (% AI)	Death criteria	LT$_{15}$ d (95% CL)	LT$_{50}$ d (95% CL)	LT$_{90}$ d (95% CL)	Regression equation $y = a + bx^a$	R^2
Hydramethylnon (0.733)	Abnormal + Moribund/dead	0.53 (0.35–0.80)	1.53 (1.01–2.32)	5.37 (3.57–8.20)	$y^b = -0.543 + 0.849x$	0.83
Hydramethylnon (0.733)	Moribund/dead	2.32 (1.20–3.43)	6.66 (5.52–7.84)	10.76 (9.10–12.50)	$y^c = 0.703 + 0.002x^d$	0.87
Indoxacarb (0.045)	Abnormal + Moribund/dead	0.43 (0.27–0.70)	0.66 (0.41–1.07)	1.12 (0.70–1.81)	$y^e = -6.804 + 5.663x$	0.76
Indoxacarb (0.045)	Moribund/dead	0.74 (0.49–1.12)	1.19 (0.79–1.81)	2.15 (1.44–3.30)	$y^e = -7.235 + 4.970x$	0.81

Lethal times were based on the criteria of either abnormal behavior plus moribund/dead ants or moribund/dead ants only. Mortality corrected by Abbott's formula. Backtransformed LTs and CLs are presented in days.

[a] x, log$_{10}$(hour).

[b] Lethal times were estimated from regression of angular transformed (arcsine$\sqrt{Y}$) data over hours 2–336 ($n = 140$).

[c] Lethal times were estimated from regression of square root-transformed ($\sqrt{Y} + 0.5$) data over hours 2–336 ($n = 140$).

[d] x, hour.

[e] Lethal times were estimated from regression of probit-transformed data over hours 2–144 ($n = 100$).

Argentine ants, *Linepithema humile* (Mayr), Rust et al. (2004) considered baits that had LT$_{50}$ values between 1 and 4 d to exhibit delayed toxicity. The above-mentioned studies also considered a range of toxicant concentrations at which the delay in toxicity can be maintained to compensate for dilution of the toxicant due to trophallaxis within a colony (Reid and Klotz 1992) and concentrating effects of evaporation in liquid baits (Rust et al. 2004). In our study, delayed toxicity followed the definition of Stringer et al. (1964) and was determined for only a single concentration of the commercially formulated baits. Based on the laboratory and field studies, the death of colonies treated with indoxacarb and hydramethylnon baits indicated that these active ingredients were being distributed throughout colonies at effective concentrations.

Descriptions of delayed toxicity vary with mode of action of the active ingredient (Reid and Klotz 1992, Klotz and Reid 1993). The abrupt change from no effect to high levels of behavioral impairment and moribundity/death displayed in the indoxacarb treatment most likely reflects the bioactivation of indoxacarb into a toxic metabolite. In lepidopteran larvae, conversion of indoxacarb to the active metabolite corresponded to the onset of neurotoxic symptoms within 4 h of oral ingestion (Wing et al. 2000). The longer delay in symptoms and death from hydramethylnon ingestion reflects its inhibition of the mitochondrial electron transport system, which causes an eventual decline in ATP production (Hollingshaus 1987). In contrast, spinosad directly stimulates nicotinic acetylcholine receptors, resulting in paralysis from neuromuscular fatigue via prolonged hyperexcitation (Salgado 1998). Spinosad effects are highly dose-dependent and have been shown to occur within 4 h in cockroaches (Salgado et al. 1998, Salgado and Saar 2004). Thus, a delay in toxicity was not evident in the spinosad bait treatment in our study.

The consistent colony mortality with indoxacarb and the nonoverlapping confidence intervals for the LT$_{15}$ and LT$_{50}$ between the spinosad and indoxacarb baits indicated that the initial 24 h delay in toxicity specified by Stringer et al. (1964) is not an absolute criterion for the selection of an effective bait toxicant and concentration. Mean cumulative mortality for indoxacarb was <2% for the first 8 h and <9% at 12 h in the delayed toxicity study. When abnormal behavior included with moribundity/death as the criteria for determining delayed toxicity, symptoms in the indoxacarb treatment were observed in only 4% of the ants at 8 h in contrast to 28% at 12 h. Because abnormal behavior plus moribundity/death account for the ants' functional ability to distribute toxicant, the very low (<5%) disruption in normal fire ant behavior for 8 h may be sufficient time to permit thorough toxicant distribution throughout an *S. invicta* colony. A response profile, where there is virtually no adverse effects on behavior for several hours and is followed by an abrupt, rapid increase in mortality, represents a desirable characteristic of active ingredients for the development of fast-acting fire ant baits. In addition, to obtain consistent efficacy, this type of response profile may need to occur over a range of dosages (Stringer et al. 1964, Williams et al. 1980, Vander Meer et al. 1985, Reid and Klotz 1992, Klotz and Reid 1993).

Because of the need to provide time for bait foraging and toxicant distribution, selection of active ingredients for fire ant baits traditionally had to meet criteria that included at least a 24-h delay before causing significant mortality. However, the efficient foraging and trophallactic behavior of *S. invicta* (Sorenson and Vinson 1981, Ferguson et al. 1996) can make it susceptible to toxicants or toxicant concentrations that have shorter delays. Exploiting the traits that make some ant species dominant arthropods in ecosystems can lead to improved methods of control. This has been illustrated by the indoxacarb bait and fire ants and perhaps can be used as a model for the development of other faster-acting ant baits.

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1748 JOURNAL OF ECONOMIC ENTOMOLOGY Vol. 99, no. 5

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