Laboratory Evaluation of Dinotefuran and Novaluron Amended Baits Against *Paratrechina* sp. nr. *pubens*[1]

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ABSTRACT With the introduction of an invasive pest ant species, *Paratrechina* sp. nr. *pubens*, it has become imperative to develop novel control technologies. There is currently no published research concerning dinotefuran and novaluron against pest ants. *Paratrechina* sp. nr. *pubens* workers and brood were exposed to baits containing dinotefuran and novaluron at varied concentrations. Liquid bait amended with dinotefuran was applied in the laboratory against *P.* sp. nr. *pubens*. Mean percent mortality of *P.* sp. nr. *pubens* was typically higher as the concentration increased at both three (F = 7.28; df = 28; $P < 0.001$) and seven (F = 7.28; df = 28; $P < 0.001$) d post-treatments. Three day observations of the lowest concentration (0.00006%) indicated a significantly lower efficacy than the highest two concentrations. LD_{50} and LD_{90} values at three and seven d post treatment showed a poor fit to the model (df = 1; $\chi^2 = 7.20$; $P < 0.01$, df = 1; $\chi^2 = 7.09$; $P < 0.01$, respectively). The use of dinotefuran was highly efficacious against *P.* sp. nr. *pubens*, and is recommended for further laboratory research and initial field research. Corn grit bait amended with novaluron was applied in the laboratory against *P.* sp. nr. *pubens* workers and brood. At four wk results did not reveal significant differences among concentrations with active ingredient and controls (F = 1.504, df = 3, 27, P = 0.239). Results of the study were inconclusive regarding the efficacy of novaluron against *P.* sp. nr. *pubens*. The findings of this study emphasize the difficulties in maintaining incomplete colonies of *P.* sp. nr. *pubens* that contain brood under laboratory settings.

KEY WORDS Dinotefuran, novaluron, *Paratrechina*, bioassay, neonicotinoids, invasive, exotic, bait, IGR

Neonicotinoids comprise a class of insecticide that is very effective against a great variety of insects. Neonicotinoids demonstrate agonistic activity on arthropod postsynaptic nicotinic acetylcholine receptor sites (Tomizawa & Yamamoto 1993, Miyagi et al. 2006). Dinotefuran, *N*-methyl-N′nitro[*N*″-[(tetrahydro-3-furanyl)methyl]guanidine, has insecticidal activity that includes both neuron-excitatory and neuron-blocking mechanisms (Kiriyama & Nishimura 2002). Dinotefuran is a 3rd generation neonicotinoid with broad spectrum activity against insects (Wakita et al. 2003). Typically known as and used in agricultural products (Elbert et al. 1998), neonicotinoids effectiveness has been further expanded to the control of urban insect pests (e.g., Premise®, Maxforce®

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Granular Fly Bait, and Advantage® (imidacloprid) for control of termites, flies, and fleas and ticks). Dinotefuran insecticidal activity has previously been demonstrated across a few insect groups including houseflies, *Musca domestica* (Kiriyama et al. 2003), mosquitoes (Corbel et al. 2004) and cockroaches (Mori et al. 2001, Kiriyama & Nishimura 2002, Miyagi et al. 2006). Neonicotinoids have proven to have a low toxicity to mammals (Kiriyama & Nishimura 2002, Corbel et al. 2004).

Novaluron, 1-[3-cloro-4-(1,1,2-trifluoro-2-trifluoro-methoxyethoxy)phenyl]-3-(2,6-difluorobenzoyl)urea, is an insect growth regulator (IGR) that has been used against a variety of arthropods (Ishaaya et al. 2003, Su et al. 2003, Cabrera et al. 2005). However, there is no published research of this IGR against formicid species. Although IGRs have adverse affects against other ant species in the laboratory (Banks et al. 1983, Kabashima et al. 2007), field control of ants using (IGR) baits can be difficult due to their temporally dynamic nutritional needs. Sustainable amounts of an IGR must be maintained within the colony and be available to the brood in an effective dose during molts. These difficulties are compounded by the inactivity of IGRs on worker and alate castes. Colony death occurs when lack of worker replacement and natural death of adult castes take place (Banks et al. 1983).

There is currently no published research concerning dinotefuran efficacy against ants. An invasive ant species, *Paratrechina* sp. nr. *pubens*, has created numerous problems in southeast Texas since 2002 (Meyers & Gold unpublished data). This invasive species has caused numerous electrical shortages of a variety of apparatuses, ecological dominance, companion animal avoidance of outdoors, and they are an immense nuisance due to their high density in urban and commercial environments (Meyers & Gold unpublished data). Since its introduction, this tramp ant has spread to 25 geographically distinct locations in five Texas counties. According to field observations from pest control operators, and preliminary laboratory studies, very few commercially available bait matrices are attractive to *P.* sp. nr. *pubens*. It is imperative to discover attractive and successful bait matrices as part of a temporally comprehensive control strategy for the management or eradication of *P.* sp. nr. *pubens* populations. Typical control tactics for urban ant pest population management of *P.* sp. nr. *pubens* have been inadequate due to its remarkable population densities. Novel control measures should be evaluated regarding population management of *P.* sp. nr. *pubens*. Successful control research tactics will likely be integrated into an overall management program for *P.* sp. nr. *pubens* control or eradication.

Invasive social insects can create ecologically devastating results (Moller 1996, Chapman & Bourke 2001, Holway et al. 2002). Social behaviors of ants create a weakness that can be exploited during the control process. Shared resources, trophallaxis, cannibalism, communication, and grooming are all avenues for an increase in treatment efficacy. This is particularly evidenced by the horizontal transmission of active ingredients (AI's), as has been demonstrated in cockroaches (Kopanic & Schal 1999), termites (Ibrahim et al. 2003) and ants (Soeprono & Rust 2004). Invasions by social insects often encompass large geographical regions, are detrimental to agricultural systems and natural communities, and are expensive to control (Vinson 1986, Vander Meer et al. 1990, Williams 1994). The ease of application of aerially applied pesticides is a desirable character for a management program for invasive species. Baits could

be integrated into an overall management program. These programs have been historically evaluated (e.g., Mirex against the red imported fire ant, *Solenopsis invicta* [Banks et al. 1973]), and more recently for termites as "Operation Full Stop" for the Formosan subterranean termite, *Coptotermes formosanus*, in New Orleans, Louisiana (Ring et al. 2001).

The use of baits for eradication of ants has been reviewed (Stanley 2004). The use of baits has proven successful against other invasive species behaviorally similar to *P.* sp. nr. *pubens*. Unicolonial ants, such as the Argentine ant, *Linepithema humile* (Krushelnycky et al. 2004), and the yellow crazy ant, *Anoplolepis gracilipes* (Abbott & Green 2007), have been successfully controlled despite high densities. Containment of an early detected invasive species may afford time to successfully manage or eradicate incipient populations (Krushelnycky et al. 2004).

These studies evaluated the biological activity of dinotefuran and novaluron against *P.* sp. nr. *pubens*. The objective for these studies was to determine mortality ratios of *P.* sp. nr. *pubens* at various concentrations of dinotefuran and novaluron amended into liquid and a corn grit bait matrices, respectively. This study constitutes an initial effort to find control alternatives for *P.* sp. nr. *pubens* in Texas.

Materials and Methods

Evaluation of dinotefuran against *P.* sp. nr. *pubens*. Each of thirty plastic boxes, 9 cm high × 15 × 30, coated with fluon, contained 100 *Paratrechina* sp. nr. *pubens* workers collected from Pasadena, TX (29°36.748 N, 95°03.313 W). Workers were collected from laboratory maintained queenright colonies of moderate size containing brood. Glass tubes, 1.6 × 15 cm were placed in each box, containing deionized water with a cotton plug. Tubes were covered with solid color construction paper for darkening purposes. Five replications at each of five concentrations of dinotefuran (0.00006, 0.00012, 0.00025, 0.0005 and 0.001%) were used, along with five replications of the product with no AI (blank). Concentrations were selected based on the suggestions provided by the manufacturer. Insecticide was provided in aqueous solution at 0.001%. All dilutions were made using 20% sucrose in deionized water. Ants were starved for 24 h prior to exposure. Two mL droplets of dinotefuran or blank were placed on the bottom of each box. Observations were made at 1, 2, 3, 4, 5, 24, 48, 72, and 168 h after application, and moribund ants were counted. By the end of the study the numbers of live ants were counted as opposed to the number of dead, as it became apparent that the ants were cannibalistic. Counts of dead or live ants were made after the 24 h starvation period and statistical analysis was conducted accordingly.

One-way Analysis of Variance (ANOVA) was used to determine significant difference in mean percent mortality in treatments. Means were separated using Tukey-Kramer HSD test. LD_{50} and LD_{90} values of mortality response to treatments were analyzed using PROC PROBIT (SAS Institute 2000, Cary, North Carolina).

Evaluation of novaluron against *P.* sp. nr. *pubens*. Novaluron was administered at various concentrations to *P.* sp. nr. *pubens* in granular form using ACAB matrix (0.1, 0.25, 0.5, and 0.0% AI). *Paratrechina* sp. nr. *pubens* were starved for 24 h pre-treatment. The colonies were allowed to feed on the bait for 1 week, after which the bait container was removed. Throughout the length of the experiment, *P.* sp. nr. *pubens* were offered 25% honey-water and crickets.

Fig. 1. *P.* sp. nr. *pubens* workers adhered to a crystallized droplet of dinotefuran.

Each replicate consisted of 100 workers and 50 brood (small egg clusters, larvae, and/or pupae). Two colonies were field-collected and laboratory-raised from which the experimental units were derived. All replicates were placed in plastic boxes, 9 cm high × 15 × 30, coated with fluon and provided glass containers fitted with water-wicks (Fig. 1). Colonies were exposed to CO_2 until movement ceased and individual workers and brood could easily be counted and removed using a camel-hair paint brush. Colonies were placed in clear Petri dishes (3.5 × 1.0 cm) containing dental stone substrate for observational purposes and moisture retention. Into the top of each Petri dish, two holes were made to allow for worker movement. Post-treatment observations of worker and brood numbers, including abnormal behaviors, were made at each time interval. Deviation from the original colony numbers were used to determine efficacy of novaluron concentrations. Each concentration of novaluron was repeated seven times. Based on previous experiments with *P.* sp. nr. *pubens* colonies, replications for this study were maintained in a growth chamber at ~29.5°C and ~64.5% humidity. All treatments and replicates were done with a completely randomized block design (CRBD).

Post-treatment counts were conducted by exposing surviving *P.* sp. nr. *pubens* to CO_2 until rapid movements ceased and workers and brood could be counted. To determine efficacy of novaluron, observations of live and dead workers and brood were made at 3, 7, 14, and 28 d post-treatment. Efficacy was determined based on the comparisons of reduction of post-treatment counts from pre-treatment counts. The evaluation of dinotefuran against *P.* sp. nr. *pubens* revealed that workers are cannibalistic. Temperature and humidity data were taken every hour throughout the experiment using a HOBO Data Logger (Onset Computer, Bourn, MA).

One-way Analysis of Variance (ANOVA) was used to determine significant difference in mean mortality (workers) and survival (larvae) in treatments (JMP, SAS Institute, Cary, NC). Means were separated using Tukey's HSD test.

Table 1. Mean dinotefuran-treated *P.* sp. nr. *pubens* mortality rates with doses using five replications of 100 ants per arena.

Concentration (%)	Mean % mortality in five replications @ 3dat[ab]	Mean % mortality in five replications @ 7dat[ac]
0.001[d]	78.82 a	89.17 a
0.0005	63.36 a	82.51 ab
0.00025	58.61 ab	88.62 a
0.00012	44.82 abc	87.61 a
0.00006	16.47 bc	57.15 b
Blank	3.31 c	4.18 c

[a]Means in the same column followed by the same letter are not significantly different ($P < 0.05$; Tukey-Kramer HSD).
[b]$F = 7.28$; df = 28; $P < 0.001$.
[c]$F = 26.57$; df = 28; $P < 0.0001$.
[d]For statistical purposes, this dose had only four replications.
dat = days after treatment.

Results

Evaluation of dinotefuran against *P.* sp. nr. *pubens*. Mean percent mortality of *P.* sp. nr. *pubens* was typically higher as the concentration increased at both three ($F = 7.28$; df = 28; $P < 0.001$) and seven ($F = 7.28$; df = 28; $P < 0.001$) d post-treatments (Table 1). There were no significant differences between the four highest concentrations for both post-treatment observations. Three d observations of the lowest concentration (0.00006%) indicated a significantly lower efficacy than the highest two concentrations. LD_{50} and LD_{90} values at three and seven d post treatment (Table 2) showed a poor fit to the model (df = 1; $\chi^2 = 7.20$; $P < 0.01$, df = 1; $\chi^2 = 7.09$; $P < 0.01$, respectively).

Evaluation of novaluron against *P.* sp. nr. *pubens*. One-Way ANOVA was conducted to determine any bias in replication placement within the growth chamber. This analysis found no bias within replications ($F = 0.38$, df = 6, 101, $P = 0.89$).

There were no statistical differences found between treatments throughout time. No statistical differences were found between means of dead workers by treatment throughout time (Table 3). At 14 d post-treatment, the only statistically significant differences ($P = 0.028$) were found between treatments of live larvae (Table 4). However, the results did not differentiate the control means from two of the AI treatments (0.1 and 0.5%). The results for both dead workers and live larvae were inconclusive.

Discussion

Evaluation of dinotefuran against *P.* sp. nr. *pubens*. Dinotefuran caused more mortality in *P.* sp. nr. *pubens* than did blank controls. With the relatively low LD_{90} values, these data indicate high efficacy of dinotefuran to control *P.* sp. nr. *pubens*. These data also indicated that dinotefuran caused sufficient mortality to warrant further testing in both the laboratory and field;

Table 2. Probit regression of mortality data to dinotefuran-treated *P.* sp. nr. *pubens* workers at different time intervals with LD values in percent active ingredient.

# replications	Days after treatment	Slope ± SE	LD_{50} (95% FL)	LD_{90} (95% FL)	χ^2
30	3	0.99 (0.37)	0.0003 (0.00008–0.0008)	0.005 (0.001–137.75)	7.20
30	7	0.84 (0.32)	1.67×10^{-5} (3.08×10^{-9}–5.53×10^{-5})	0.00055 (0.00025–0.037)	7.09

Table 3. Mean # of dead *P.* sp. nr. *pubens* workers throughout time treated with novaluron using Advance Carpenter Ant Bait matrix amended with novaluron.

	Mean (SE ±) # of dead workers throughout time (d)[a]			
Treatment (AI%)	3[b]	7[c]	14[d]	28[e]
0.10	9.14 (3.13) a	21.14 (3.26) a	32.29 (5.68) a	55.14 (4.74) a
0.25	6.86 (3.13) a	14.43 (3.26) a	22.00 (3.35) a	41.14 (5.49) a
0.50	4.57 (0.95) a	20.86 (5.39) a	32.00 (6.39) a	53.43 (6.3) a
0.0 (Control)	9.00 (3.18) a	22.29 (5.81) a	32.57 (5.37) a	59.00 (8.03) a

[a]Means with same letter in the column are not significantly different ($P < 0.05$; Tukey's HSD).
[b]$F = 0.818$, df = 3, 27, $P = 0.497$.
[c]$F = 0.644$, df = 3, 27, $P = 0.595$.
[d]$F = 0.937$, df = 3, 27, $P = 0.438$.
[e]$F = 1.504$, df = 3, 27, $P = 0.239$.

however, the delivery system of dinotefuran will need modification for field tests. Applying this bait matrix with corn grit or other food substances may decrease evaporation and crystallization rate, along with increasing the likelihood that workers will be able to allocate the bait to remaining colony members.

High survival ratio within the control replications suggests an unbiased analysis of the experiment. However, extraneous factors such as crystallization (Fig. 1) of dinotefuran, and cannibalism may have affected mortality in this no-choice test. Crystallization of the bait may not have allowed for continued feeding past ca. 48 h (Fig. 1). Some individuals became adhered to the product and therefore died *in situ*, which may have adversely affected spread of the insecticide throughout the remaining workers. Crystallization may have caused a differential availability of dinotefuran within the formulation. The primary dissipation route for dinotefuran may be through aqueous photolysis (~1.3 d). Sorting and

Table 4. Mean # live *P.* sp. nr. *pubens* larvae throughout time treated with novaluron using Advance Carpenter Ant Bait matrix amended with novaluron.

	Mean (SE ±) # of live larvae throughout time (d)[a]			
Treatment (AI %)	3[b]	7[c]	14[d]	28[e]
0.10	20.71 (1.52) a	6.71 (1.29) a	3.86 (1.18) ab	0.17 (0.17) a
0.25	23.29 (1.69) a	7.57 (1.09) a	1.57 (0.65) b	0.00 (0) a
0.50	20.43 (1.88) a	9.00 (1.42) a	5.86 (1.96) ab	1.00 (0.45) a
0.00 (Control)	27.00 (2.04) a	8.71 (1.51) a	7.29 (1.11) a	0.83 (0.65) a

[a]Means with same letter in the column are not significantly different ($P < 0.05$; Tukey's HSD).
[b]$F = 2.894$, df = 3, 27, $P = 0.056$.
[c]$F = 0.628$, df = 3, 27, $P = 0.604$.
[d]$F = 3.589$, df = 3, 27, $P = 0.028$.
[e]$F = 1.620$, df = 3, 27, $P = 0.216$.

separation of the dead individuals from the living group of workers would not have allowed for the opportunity of cannibalism. This cannibalistic behavior towards exposed individuals of social insects increases the transmission of an insecticide throughout the population (Kopanic & Schal 1999, Ibrahim et al. 2003, Soeprono & Rust 2004). Given the relative stability of dinotefuran, this is likely the case regarding its interaction with *P.* sp. nr. *pubens* both physiologically and behaviorally. It is unknown whether *P.* sp. nr. *pubens* workers were cannibalistic toward healthy or moribund workers, or simply consume cadavers as part of a normal behavioral assemblage. Although no counts were taken of major body parts (head, thorax, or abdomen), observations indicate that consumption of the head was considerably less likely than the thorax or abdomen. Further studies on horizontal transfer of insecticide through cadaver maintenance or cannibalism should be investigated in *P.* sp. nr. *pubens*. Metabolic dissipation pathways of dinotefuran should also be investigated. These findings may indicate the reasoning for high horizontal transmission through behaviors (trophollaxis, grooming or other) or cannibalistic insects.

The relative success of this laboratory study warrants further laboratory evaluations and initial field efficacy investigations. These findings may assist pest control operators during their efforts to control the numerically superior pest.

Evaluation of novaluron against *P.* sp. nr. *pubens*. Despite a supposed ideal environment of temperature and humidity (29.45°C ± 0.007, 64.57% ± 0.09, respectively), workers and brood of replications began dying at a surprising rate. Because of this, the original experiment was cancelled and performed again. The initial experiment was run under the same parameters (with exception of 100 brood rather than 50) and was considered inconclusive. Statistical analyses were conducted on the truncated data, and no apparent biases were found within the experiment. We believe that this demonstrated the difficulties in maintaining *P.* sp. nr. *pubens* in colony-form with low worker numbers and without the presence of queens.

Paratrechina sp. nr. *pubens* provisioned bait granules (both control and AI) (Figs. 2 & 3). The workers placed the bait inside the Petri dish, or upon and around the water-wick. This created an ideal environment (high moisture) for fungal growth. These facts may have hampered the ability to perform a more informative test; however, if statistical differences were to be found, they would have likely occurred at greater than 28 d post-treatment.

Formicid species often demonstrate temporal fluctuation of food resource consumption. This is not an ideal situation for IGR efficacy testing. For efficacy of IGRs to be expressed, there needs to be enough titer within a specific time interval (i.e., during larval molt). Because formicids select alternative food resources throughout time, administering an IGR can be a difficult task. Nevertheless, based on these results, this product could not be used for the control of *P.* sp. nr. *pubens*; however, additional laboratory and field research needs to be done. The laboratory studies should include whole colony tests with natural ratios of brood workers and queens. If used in the field, it would likely be most effective to broadcast large quantities of the bait during early spring as large numbers of brood are maturing.

Although not supported from these results, there remains the possibility that novaluron is effective against *P.* sp. nr. *pubens*. Another study conducted during experiments with red imported fire ant, *Solenopsis invicta*, found that methoprene was not effective against another *Paratrechina* sp. (Sanchez 2005).

Fig. 2. This picture demonstrates the provisioning of bait and subsequent fungal growth associated with the high humidity and the clustering behavior of *P.* sp. nr. *pubens*. The discoloring (yellowing) of the wick seen here is typical of all field-collected colonies maintained in the laboratory.

In that study, an increase in *Paratrechina terricola* populations were found in trees located in areas treated with methoprene.

A previous experiment (Meyers et al., unpublished data), field observations, and communication with various pest control operators with clientele affected by *P.* sp. nr. *pubens*, suggested that the current label rate for ACAB (abamectin) is not effective. The currently recommended rate of 1.5 lbs per acre is unlikely to create or sustain control of the numerically dense *P.* sp. nr. *pubens* populations. If an additional AI was integrated into the product, or an increase in the current broadcast rate, the efficacy of ACAB may increase. If additional bait amounts were used, the efficacy of the product would likely increase. The field effectiveness of this product at current label and expanded usage should be assessed against *P.* sp. nr. *pubens* in early spring.

P. sp. nr. *pubens* are considerably attracted to the ACAB matrix in the laboratory and field. It is therefore recommended that ACAB with novaluron be tested against large laboratory colonies (with a full compliment of castes). Field observations suggest an immense increase in numbers of *P.* sp. nr. *pubens* brood and worker members during early spring. During this period foraging for food sources high in protein is needed for brood production. ACAB contains a marine lipid based attractant. Therefore, this product may be a viable option as part of a temporally dynamic control program against *P.* sp. nr. *pubens*.

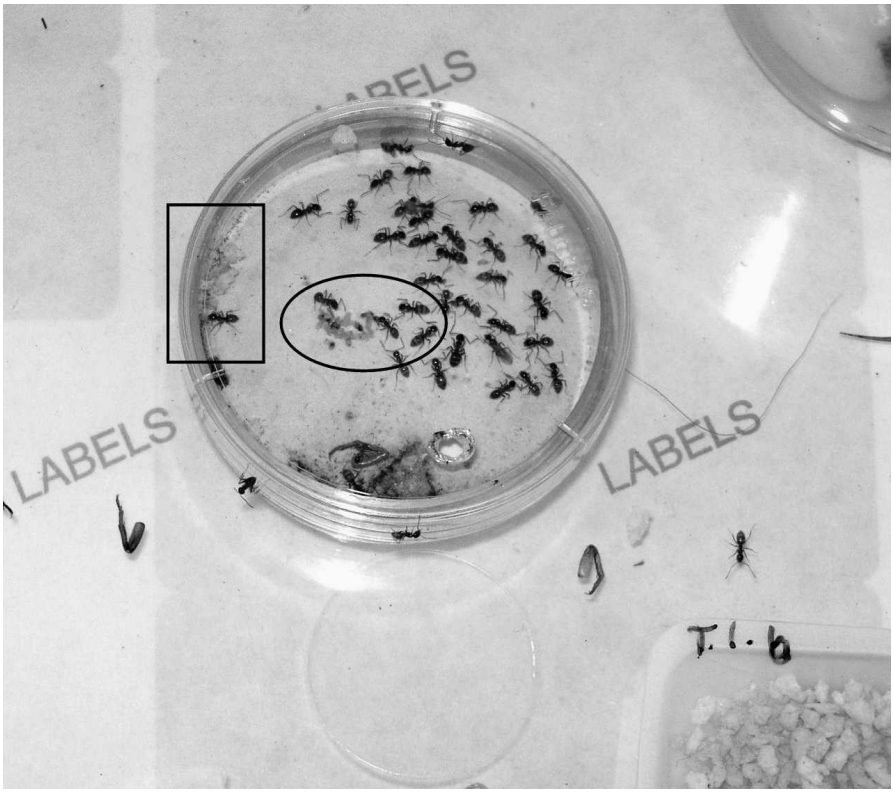

Fig. 3. This picture demonstrates the provisioning of the bait inside the Petri dish. The square shows provisioned bait granules for 0.1% AI treatment. The circle shows workers tending several larvae.

The results of this laboratory study underscores the difficulties of maintaining relatively small, queenless colonies of *P.* sp. nr. *pubens*. Although it is not known whether the lack of queens adversely affected the outcome of the study, it could be one of the contributing factors.

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