Recommendations for Treated-Area Choice Assays with Termites (Isoptera)

by

Chris J. Peterson[1], Patrick D. Gerard[2], & Janice Ems-Wilson[3]

ABSTRACT

The repellency of catnip (*Nepeta cataria*) essential oil was evaluated in a treated-area choice assay with subterranean termites (*Reticulitermes* spp.). It appeared that fewer *R. virginicus* were found on the treated portion of a Petri dish within a period of about 7 d; *R. flavipes* was not affected by the presence of the oil. The data collected from the control dishes, however, showed an unacceptably high Type I error rate (rejection of H_0: $n_t = n_u$, when H_0 is true, where n_t is the expected number of termites on the treated side and n_u is the expected number of insects on the untreated side). The tendency of termites to cluster was the probable reason for this, and calls into question the data obtained from the tests. Computer simulations, using a range of cluster factors, replications and numbers of termites per replication, were conducted, and the Type I error rate was calculated when a cluster center (a point corresponding to a random angle θ and radius ρ value within a circle) and random "termite" positions about the cluster center were plotted. Type I error rates were consistently inflated for all analyses that were based on individual animal behavior, and could not be corrected by increasing replications. We recommend an analysis where the number of insects on the treated or untreated side is recorded, then the dish is designated as "repelled" or "not repelled," and the number of repelled dishes is analyzed. This method effectively controls the Type I error rate so that it is no greater than the nominal value. It is recommended to use at least 25 replications to ensure adequate statistical power. Analysis of these types of data is best accomplished by use of Fisher's Exact test or Boschloo's Exact Unconditional test.

KEYWORDS: *Reticulitermes* spp., choice-assay, repellent, clustering, analysis

---

[1]USDA Forest Service, Wood Products Insect Research Unit, 201 Lincoln Green, Starkville, MS 39759 USA

[2]Experimental Statistics Unit, Mississippi State University, Mississippi State, MS 39762 USA

[3]Department of Chemistry, Valencia Community College, Orlando, FL 32802 USA

INTRODUCTION

Measuring repellency accurately and reproducibly is of paramount importance to the development of repellent pest control products. Before a compound goes to costly, risky and time-consuming field trials, reliable laboratory tests are required to screen out compounds of low potential. The choice-test assay is very popular in entomology. In such an assay, the insects have equal access to two similar situations, differing only in the presence or absence of a treatment. The treatments may consist of a treated substrate or a treated food source, depending on the test, and record is made of any number of endpoints, such as the number of seconds the insect spends on each side (Peterson *et al.* 2002), the amount of food eaten on each side (Oi *et al.* 1996), or the number of insects on each side (Sbeghen *et al.* 2002). The general theme takes on many variations, but the analysis of data relies on the assumption that in the absence of a repellent or attractive stimuli (as in control groups), the insects will respond to each choice equally and independently of one another.

Results obtained by us and presented here, however, indicate that the distribution of insects within a choice arena under control conditions is not random, thereby casting doubt upon the ability to determine treatment effects. Similar observations were made by Delaplane & La Fage (1987) and Oi *et al.* (1996). The possible reasons for non-randomness are many. It is known, for example, that termites produce trail pheromones (Howard *et al.* 1976), and termites are often observed in a head-to-tail line. Thus, the distribution of insects is often clustered, both in space and time. Termites should not be expected to distribute uniformly throughout a test apparatus, and any endpoints based on the location or numbers of termites within the apparatus may be invalidated by clustering. Nevertheless, choice assays are commonly seen in the literature with no consideration of clustering effects, despite the observations and recommendations of previous researchers. Delaplane & La Fage (1987) noted non-randomness (clustering) in feeding on wood blocks, and recommended that seven experimental units be used for power = 0.95 at $\dot{\alpha} = 0.05$ based on deviation of means from the grand mean. Oi *et al.* (1996) also report clustering in a feeding study, but conclude that the nonrandom nature of termite feeding invalidates standard sample size calculations based on variance, and that "enough replicates" need to be used "so that controls are not significantly different."

In this study, we report the results of a termite repellency assay with catnip oil conducted in the summer of 2002. Computer simulations of

choice assays are used to demonstrate how clustering interferes with the analysis of experimental results by inflating the Type I error rate. We therefore propose an experimental design and data analysis that provides improved statistical properties.

METHODS AND MATERIALS

Termites

Subterranean termites (*Reticulitermes* sp.) were collected in June 2002 from the Choctaw Wildlife Management Area of the Tombigbee National Forest near Ackerman, MS USA. Two populations, separated by 3 km, were collected from infested pine logs and taken to the laboratory for identification. Soldiers (alates were not available) were identified by using the keys of Gleason and Koehler (1980) and Scheffrahn and Su (1994) as *Reticulitermes virginicus* (Banks) and *R. flavipes* (Kollar). The logs were stored in metal cans with lids, and termites were removed from the logs as needed throughout the test.

Catnip essential oil

The essential oil of catnip (*Nepeta cataria* L.) was purchased from Kong Pet Products, Golden, CO USA, and consisted of 98% nepetalactone, with an isomer ratio of 36: 64 *E,Z-*: *Z,E*-nepetalactone (Peterson & Ems-Wilson 2003).

Repellency assay

This assay was based on that reported by Zhu *et al.* (2001). For chemical treatments, sand was treated to constitute 0 (acetone only), 100, 250 and 500 ppm (by mass) catnip essential oil. Sand was treated by applying an acetone dilution of the catnip oil to 100 g of sand, and then placing on a jar roller for five minutes. The sand was poured into glass Petri dishes and the acetone evaporated for one hour in a fume hood at ambient temperature. One ml of agar solution was added to a 5-cm diameter by 1 cm high Petri dish. After the agar solidified, one-half of the surface of the agar was covered with 0.5 g treated sand, and the other half was covered with 0.5 g untreated sand. A piece of untreated filter paper (1 cm in diameter) was placed on each half of the dish to provide food for the termites. Ten worker termites were placed in the center of each dish, lids were placed on the dishes and secured with Parafilm® (American National Can Co., Chicago, IL USA), and then the dishes were placed in an incubator at 25°C and 70% RH in the dark. A random number table was used to determine the position of the treated and untreated sides (to the right or the left). Readings were taken every 15 min for 1 h, then hourly for the next 5 h, then every 24 h for 24 d. Dead and moribund termites were counted, and when 70% of the

termites in any dish were dead or moribund, that dish was discarded. The test had five replications.

Percentage repellency was calculated according to Sbeghen *et al.* (2002), where the number on termites on the treated side is subtracted from the number on the untreated side, then divided by the total number of insects present, then multiplied by 100 to get a percentage.

Computer simulations

All computer simulations were conducted using SAS software for Windows version 8.2 (SAS Institute 2001). In our simulations depicting clustering, a "cluster center" was chosen by the computer at random θ and ρ (angle and radius) from 0 to 360 degrees, and 0 to 1 units, respectively. Points, or "termites," were plotted randomly around the cluster center. A "cluster factor" was added to the simulation by fixing the maximum distance a termite could be found from the cluster center; higher values indicated shorter distances, and therefore more clustering. Those points falling on one side of the midline were considered "repelled" while those falling on the other side were considered "not repelled." We plotted one cluster center per dish. The simulations ran 1000 times.

In our first simulations, we determined the number of termites on each side of the dish in the described simulations. A binomial test for proportions was used to determine the Type I error rate (percentage of times rejecting H_o when H_o is true) for the null hypothesis H_o: $n_t = n_u$, where n_t is the expected number of termites on the treated side and n_u is the expected number of termites on the untreated side, and assuming equal sample sizes between dishes, in 1000 simulations. In an appropriate test, we expect the Type I error rate to be less than 5% ($\hat{\alpha} = 0.05$, two-tailed analysis). Here there were no treatment effects, i.e. the expected results in the control groups. A number of different combinations of cluster factors, numbers of dishes (replications) and numbers of termites per dish were simulated.

In the second simulations, we compared the number of termites on the untreated sides of "active" and "inactive" dishes, and calculated Type I error rates. This was more reflective of how tests are run by experimenters, where treatment groups (active dishes) are compared to control groups (inactive dishes). Of course, the "untreated" side of an inactive dish is arbitrary, because there is no treatment on either side. Here, we tested the null hypothesis H_o: $n_{ua} = n_{ui}$, where n_{ua} is the expected number of termites on the untreated side of active dishes and n_{ui} is the expected number of termites on the untreated side of inactive, or control, dishes, and assuming equal sample sizes. We did not include any treatment effects in this test, so we were comparing two sets of

control dishes to each other. In an appropriate test, we would expect the Type I error rate to be less than 5% ($\dot{\alpha} = 0.05$, two-tailed analysis) by Fisher's Exact test, because two sets of untreated dishes should have similar results. A number of different combinations of cluster factors, numbers of dishes (replications) and numbers of termites per dish were simulated.

In the next simulations, the dish was the experimental unit, rather than the termite. We considered a dish "repelled" if the number of termites on the untreated side was greater than one-half of the total number of termites per dish. Again, we compared a group of "active" dishes to a group of "inactive" dishes, and the number of repelled dishes was analyzed. The null hypothesis H_o: $d_a = d_i$, was tested, where d_a is the expected number of repelled dishes in the active group and d_i is the expected number of repelled dishes in the inactive group, and assuming equal sample sizes. We did not include any treatment effects, again simulating a comparison of two groups of control dishes. Fisher's Exact test was used to determine the Type I error rate, and we expect the rate to be less than 5% ($\dot{\alpha} = 0.05$, two-tailed analysis). A range of cluster factors, numbers of dishes (replications) and numbers of termites per dish were used in the simulations.

In addition to maintaining the nominal Type I error rate, hypothesis tests should have adequate power; that is, the tests should have a high probability of rejecting the null hypothesis when the null hypothesis is false. In actual assays, a progressively stronger repellent or attractant would progressively increase the probability of a termite (or cluster of termites) occurring on a specific side of the dish. In our final simulations, a "probability factor" was added to simulate treatment effects and evaluate the power of hypothesis tests. A probability factor of 0.5 indicated no treatment effects (an equal likelihood of a cluster center falling on the untreated side; H_o true), and values of 0.6, 0.7, 0.8 and 0.9 indicated a progressively stronger "repellent," i.e. a higher probability of the cluster center falling on the untreated side (H_o false). Fisher's Exact test was used to determine power, or the likelihood of rejecting H_o: $d_a = d_i$ when H_o is false (a correct decision). When treatment effects are introduced, we expect power to increase. We used several different cluster factors, numbers of dishes (replications) and numbers of termites per dish.

RESULTS & DISCUSSION

Repellency assay

Based on average repellency values over five replications, it appeared that catnip oil was a strong repellent to *R. virginicus*, especially in the

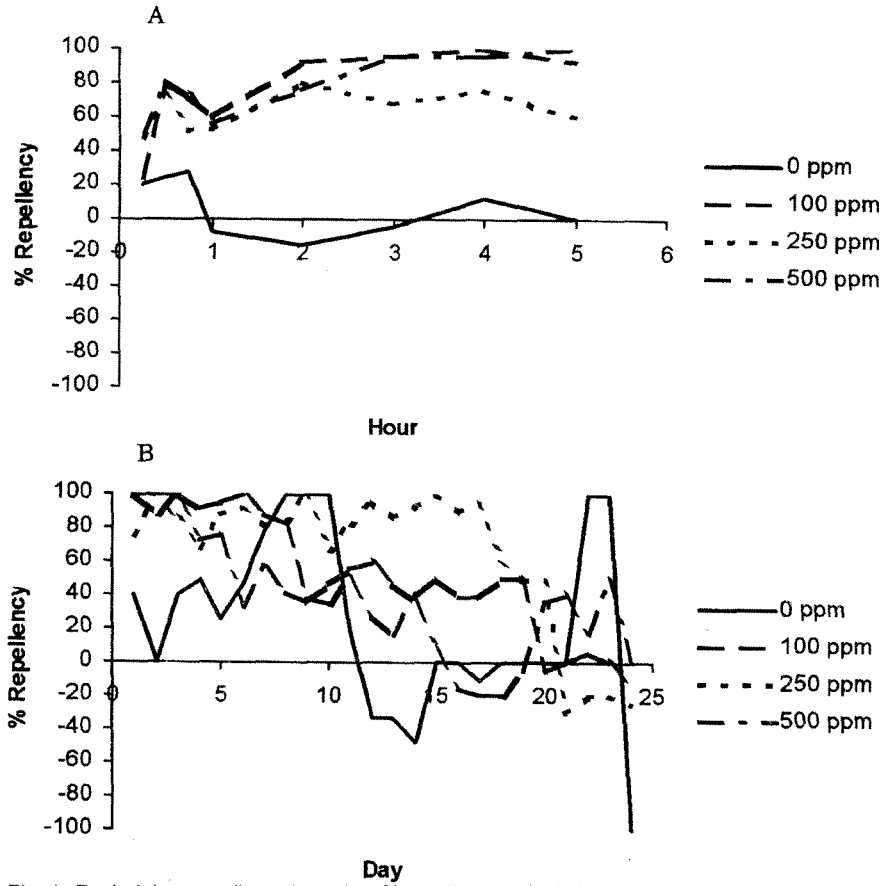

Fig. 1. *R. virginicus* repellency based on % repellency calculations (averaged over all replications) for A) the first day and B) days 1 to 24.

first 5 days (Figs. 1A and 1B). Repellency, however, began to be seen in the control group on day 8 of the test. When the individual dishes were plotted for the first day (Fig. 2A) and days 1 – 24 (Fig. 2B) of the control group, clustering of termites to one side of the dish or the other, and averaging the replications, falsely gives the appearance of uniformity. Percentage repellency values with absolute values of 40 to 45 (about seven out of 10 termites on either side) and greater are considered different from a percentage repellency value of zero (five termites to a side) in some analyses (e.g., Zhu *et al.* 2001). In the control, this is observed in one out of five replications at the 15 minute time point, four out of five at 30 min, five out of five at 45 min, four out of five at one hour, etc (Figs. 2A and 2B).

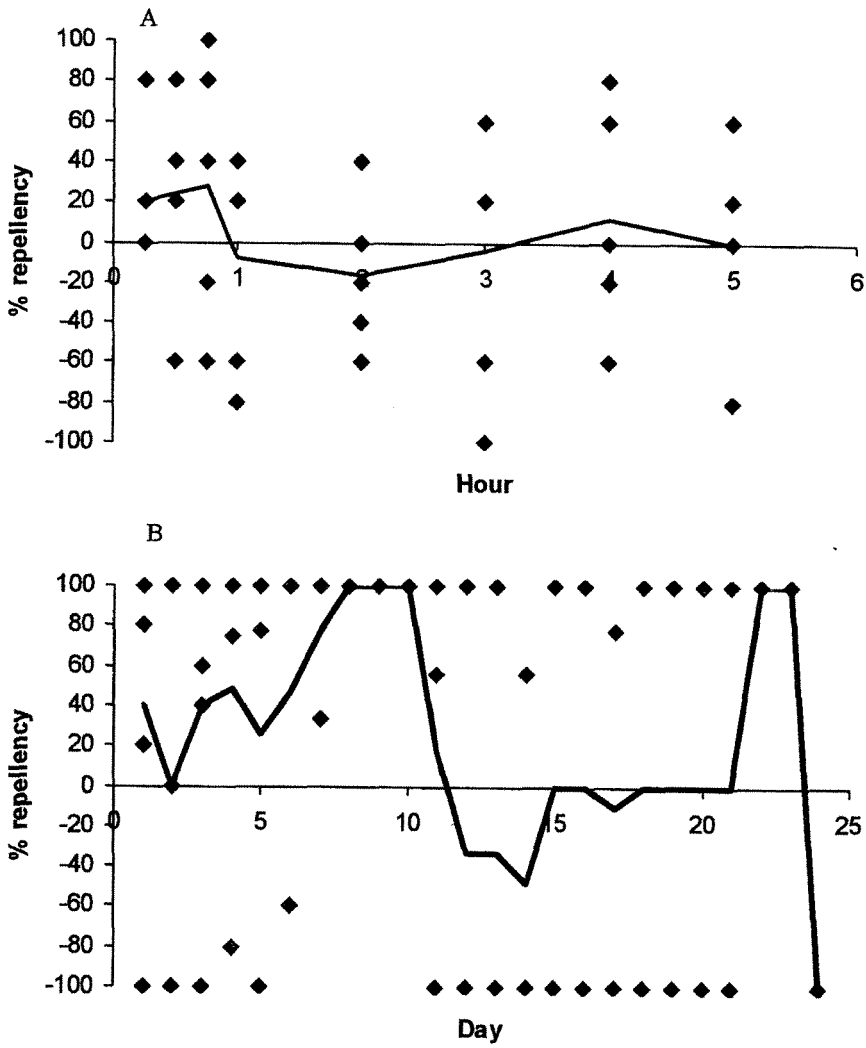

Fig. 2. *R. virginicus* control dishes showing the values of individual dishes (five replications) for A) the first day and B) days 1 to 24. The solid line is the average of all replications.

We examined the control groups of both *R. virginicus* and *R. flavipes* from the bioassay by using binomial tests. For *R. virginicus*, 50.6% of the control dishes had significantly more termites on one side than the other, and for *R. flavipes* this number was 23.2%. Because we expect a valid test to reject less than 5% of the time, it is clear that the analysis as described above is faulty.

Table 1. Type I error rates (two-tailed) when counting the number of termites on the untreated side, by using differing numbers of replications, termites per dish and cluster factors in computer simulations.

	Dishes (Replications) = 5 Termites/Dish					Dishes (Replications) = 10 Termites/Dish					Dishes (Replications) = 25 Termites/Dish				
C. F.	5	10	15	25	50	5	10	15	25	50	5	10	15	25	50
0	0.0	2.2	3.7	5.0	4.1	0.0	1.7	3.3	4.2	2.7	0.0	2.3	4.4	4.2	3.9
0.1	5.2	3.5	7.1	9.2	13.7	4.4	4.8	6.9	7.5	11.4	5.7	5.8	8.9	9.0	13.1
0.25	5.8	8.3	13.8	19.7	31.4	5.3	6.8	11.5	20.3	28.7	8.6	11.3	13.5	20.1	30.4
0.5	11.5	17.1	23.5	34.7	51.1	8.8	17.9	26.0	36.9	49.3	12.5	20.0	28.9	35.0	49.0
1	19.7	30.4	38.6	54.2	63.3	14.8	28.9	39.9	52.7	61.8	21.1	33.1	43.5	51.7	63.3
5	36.0	46.3	54.5	68.6	78.8	30.4	43.6	53.4	67.1	77.5	30.8	48.5	59.2	69.1	74.0
10	35.3	43.1	50.7	76.7	83.1	32.8	44.6	61.5	68.3	75.7	36.2	53.5	60.5	67.2	76.3
15	36.4	41.3	45.8	81.9	88.4	35.0	44.5	60.7	70.5	78.3	37.0	52.1	58.0	65.5	77.7

C.F. = Cluster Factor

Simulations

In our first simulations, we mimic the analysis run on the control dishes above. We determined the Type I error rate for H_o: $n_t = n_u$, where n_t is the expected number of termites on the treated side and n_u is the expected number of termites on the untreated side. In simulations with no clustering, the Type I error rate was 0 to 5%. Type I error rate went up with cluster factor (Table 1), approaching 90% in cases of high clustering and high numbers of termites per dish. Adding more termites to the dishes increased the Type I error rate at all cluster factors and numbers of dishes, while increasing the number of dishes (replications) had no effect. The increase in Type I error rate with an increase in the number of termites per dish is due to the fact that the binomial test assumes independence of the observations, but because of clustering the observations are not independent; the binomial test is "fooled," leading to an inflated Type I error rate. Fig. 3 shows the relationship of cluster factor to Type I error rate for ten termites per dish and five dishes as was the case in our test. Below a cluster factor of 1, the relationship was linear, with $r^2 = 0.9937$. We can then solve for cluster factor (X), and find that the 23.2% Type I error rate for *R. flavipes* corresponds to a clustering value of 0.73. For *R. virginicus*, because the observed Type I error rate of 50.6% is in the plateau region of the curve, a lower bound estimate of 2.5 for the cluster factor is reasonable.

In the second set of simulations, we compared the number of termites on each side of the dish between "active" dishes and "inactive"

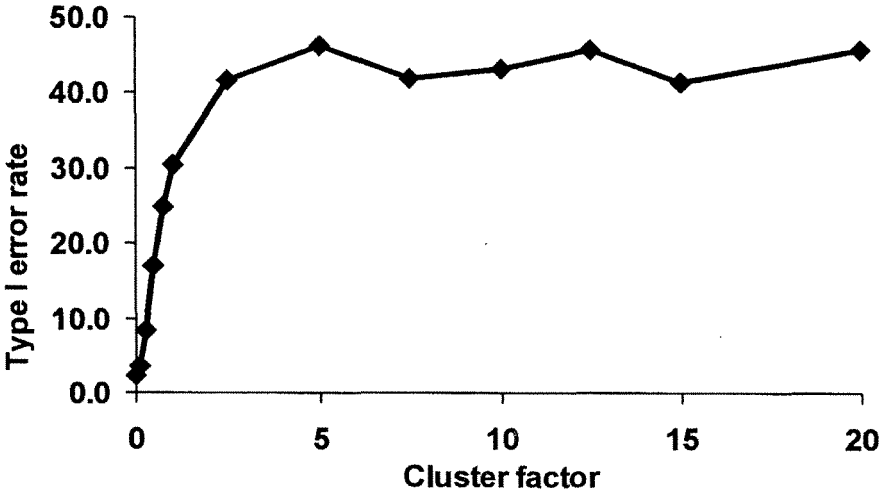

Fig. 3. Type I error rate as a function of cluster factor for 5 dishes with 10 termites per dish.

dishes. We did not include any treatment effects, so effectively we were comparing independent sets of control dishes to evaluate the Type I error rate. If the difference between two sets of control dishes is significant, then the null hypothesis (H_o: $n_{ua} = n_{ui}$, where n_{ua} is the expected number of termites on the untreated side of active dishes and n_{ui} is the expected number of termites on the untreated side of inactive, or control, dishes) is rejected. Similar to the first simulation, the Type I error rate increased as cluster factor increased, and a lesser increase was noted when the number of termites per dish increased. Increasing the number of dishes, i.e. increasing the number of replications, had no noticeable effect. The Type I error rate approached 80% in some cases (Fig. 4).

In both sets of simulations, the number of termites on a particular side were counted and termites were taken as the experimental units. The clustering (which we cannot control in actual repellency tests) caused the Type I error rate to be unacceptably high. This was aggravated by increasing the number of termites per dish, and Type I error rate could not be lowered by increasing replications. With this being the case, any results obtained by use of this type of analysis are in question, and may lead to concluding that a test compound causes repellency when, in fact, it does not.

In order to perform hypothesis tests that maintain the nominal Type I error rate, we needed a different way to express and analyze the data. If the dishes rather than the termites were taken as the experimental

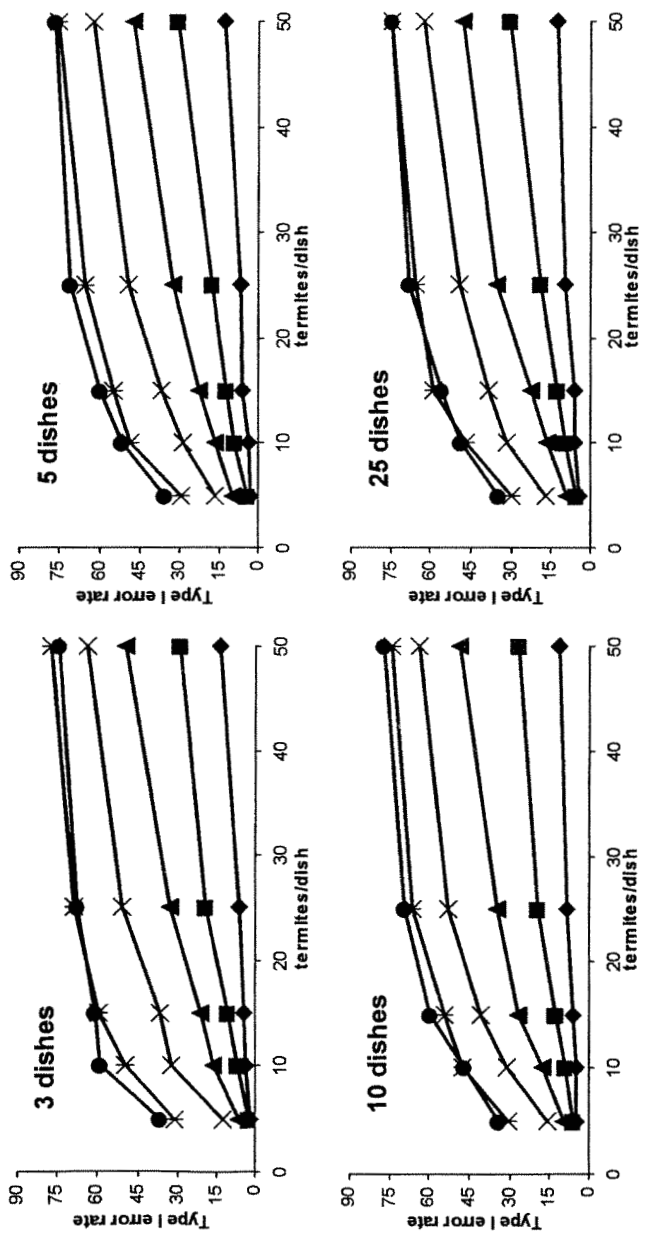

Fig. 4. Comparing the number of termites on the untreated sides of "active" to "inactive" dishes with no treatment effects. Type I error rate as a function of termites per dish for different cluster factors and numbers of dishes (replications).

unit, perhaps the clustering would not interfere with analysis. Our next set of simulations addressed this. If the number of termites on the untreated side was greater than one-half of the total number of termites

Table 2. Type I error rates (two-tailed) for the recommended analysis with 25 termites per dish, at different cluster factors and number of dishes in computer simulations.

Termites/Dish = 25

C. F.	Dishes (replications) 5	10	25
0.1	1.90	1.00	3.40
0.25	1.80	1.40	2.30
0.5	1.90	1.40	3.00
1	1.90	1.30	3.60
5	2.40	0.80	3.30
10	1.40	1.00	3.60

C.F. = Cluster Factor

present, then the dish was considered "repelled." The number of repelled dishes can be compared between "active" and "inactive" groups. Two-tailed analysis was not possible with only three dishes but was for five or more. Fisher's Exact test was used to test the null hypothesis (H_o: $d_a = d_i$, where d_a is the expected number of repelled dishes in the active group and d_i is the expected number of repelled dishes in the inactive group). There was no increase in Type I error rate with an increase in cluster factor, nor with an increase in the number of termites per dish. There was, however, a slight increase when more dishes were used (i.e. more replications), but this value did not surpass 5%, even with 25 dishes (Table 2).

With an acceptable Type I error rate, we conducted power calculations for this type of analysis. Power is the likelihood of rejecting H_o when H_o is false (a correct decision). Treatment effects were simulated by adding a "probability factor" that assigned a cluster center to the untreated side of the dish with the specified probability. In actual bioassays, a progressively stronger repellent would progressively increase the probability that the termites would move to the untreated side of a dish. The number of dishes (replications) had the greatest effect on power within any given probability factor (Table 3). The number of termites per dish had an effect as well, but to a lesser degree. Cluster factor also increased power slightly, especially with stronger "repellents." However, we cannot control clustering (and we have assumed that clustering is independent of the number of termites per dish, see Delaplane & La Fage (1987)).

It is apparent from our simulations, as well as from the analysis of our study data, that tests of statistical hypotheses that treat individual animals as behaving independently may be adversely affected by clustering, resulting in inflated Type I error rates. This fact casts doubt on any significant differences found by using such procedures. In the event that clustering is present, it is imperative that analysis methods account for this clustering, and the simplest approach entails using the dish, rather than the individual animal, as the experimental unit.

We recommend an analysis where the number of termites on each side is counted, then the dish designated as "repelled" or "not repelled."

Table 3. Power calculations (two-tailed) for the recommended analysis with treatment effects for various numbers of dishes, termites per dish and cluster factors in computer simulations

Probability = 0.6	Dishes (replications) = 5 Termites/dish					Dishes (replications) = 10 Termites/dish					Dishes (replications) = 25 Termites/dish				
C.F.	5	10	15	25	50	5	10	15	25	50	5	10	15	25	50
0.1	0.46	2.33	1.76	0.91	2.41	0.51	1.90	1.00	1.60	1.50	2.90	2.00	3.20	3.10	4.80
0.25	0.56	2.81	1.63	2.42	1.61	1.31	1.30	1.50	1.80	2.00	2.10	3.30	3.40	3.40	4.40
0.5	1.07	2.11	2.63	1.91	2.10	1.31	1.80	1.40	2.10	1.80	2.40	5.30	4.70	5.60	6.00
1	1.74	2.22	2.41	3.50	1.80	1.40	2.00	1.60	2.10	1.80	4.80	5.70	6.40	6.00	6.00
5	2.82	2.60	2.51	2.51	1.90	1.80	2.20	2.40	2.90	1.80	6.10	7.50	6.70	6.70	6.50
10	2.72	2.41	2.30	2.00	2.60	2.30	2.10	1.80	1.80	1.60	7.30	7.30	6.00	6.20	8.00
15	1.91	2.51	2.31	2.31	2.92	2.60	2.30	1.90	1.30	2.40	8.10	6.60	6.20	7.30	6.80
Probability = 0.7	Dishes (replications) = 5 Termites/dish					Dishes (replications) = 10 Termites/dish					Dishes (replications) = 25 Termites/dish				
C.F.	5	10	15	25	50	5	10	15	25	50	5	10	15	25	50
0.1	0.47	2.23	2.23	1.32	2.52	0.51	1.30	1.80	1.30	1.70	2.40	3.80	3.50	3.50	6.60
0.25	0.80	2.23	1.74	1.83	3.01	0.61	2.10	2.00	2.10	3.50	3.20	4.80	7.40	8.10	13.10
0.5	0.96	2.63	2.23	2.92	3.02	2.01	1.80	3.40	4.30	4.50	4.90	8.70	9.20	10.70	14.60
1	1.35	2.42	2.52	3.54	2.81	1.90	3.10	4.70	3.90	5.10	7.90	12.40	13.30	15.40	18.10
5	3.96	3.92	4.14	3.41	4.01	4.40	4.00	3.50	4.20	5.20	15.20	20.80	19.70	21.60	21.60
10	4.44	3.63	4.32	4.82	4.74	5.60	3.80	4.30	4.30	6.10	21.40	21.00	20.10	24.00	21.90
15	5.15	3.54	4.02	4.62	4.32	4.90	5.00	5.20	4.90	6.40	21.30	21.50	21.60	21.30	22.80

Even so, it would require at least 25 replications per each treatment and the control to ensure adequate statistical power, and even this is insufficient for weaker repellents (or lower doses). It can be argued, of course, that a repellent that increases the probability of a "cluster center" falling on the untreated side of a dish by only 10 or 20% would

Table 3 (continued). Power calculations (two-tailed) for the recommended analysis with treatment effects for various numbers of dishes, *termites per dish and cluster factors in computer simulations*

Probability = 0.8

	Dishes (replications) = 5 Termites/dish					Dishes (replications) = 10 Termites/dish					Dishes (replications) = 25 Termites/dish				
C.F.	5	10	15	25	50	5	10	15	25	50	5	10	15	25	50
0.1	0.23	2.33	0.83	2.45	3.71	1.43	1.40	2.40	2.50	3.00	3.00	3.90	4.50	6.20	11.00
0.25	0.92	2.03	1.97	3.26	4.33	1.52	3.00	2.10	3.50	6.51	4.70	10.90	11.30	17.30	25.10
0.5	0.89	3.55	4.40	4.57	4.95	1.94	4.90	5.50	8.30	7.70	9.30	18.80	21.50	24.00	33.20
1	1.80	4.26	3.99	6.77	5.37	5.03	7.30	8.60	8.80	9.40	18.70	28.50	33.70	34.80	43.30
5	6.25	8.83	6.76	6.50	5.75	9.01	11.40	11.80	11.20	12.20	39.60	42.30	42.20	49.10	47.70
10	5.78	7.09	8.33	8.35	8.24	10.70	10.20	11.60	11.20	13.30	45.00	46.40	44.30	44.70	47.70
15	5.75	6.65	6.39	6.78	7.15	12.41	12.10	13.10	13.41	11.80	46.80	49.90	46.80	49.80	49.10

Probability = 0.9

	Dishes (replications) = 5 Termites/dish					Dishes (replications) = 10 Termites/dish					Dishes (replications) = 25 Termites/dish				
C.F.	5	10	15	25	50	5	10	15	25	50	5	10	15	25	50
0.1	0.47	1.93	1.26	2.16	2.75	0.61	1.20	1.50	3.00	4.20	4.20	6.20	7.70	10.60	18.20
0.25	0.60	3.10	2.10	3.73	6.09	0.93	4.30	5.11	7.93	10.50	9.60	16.50	22.20	31.80	41.30
0.5	0.79	3.58	4.34	5.03	8.46	3.05	7.41	12.00	12.31	17.50	19.80	33.20	41.30	49.90	60.10
1	2.26	6.15	7.06	8.93	9.84	7.07	13.90	18.12	18.72	20.30	36.90	54.20	62.00	62.90	67.40
5	8.54	10.42	9.87	10.25	12.55	22.92	25.40	25.03	24.50	27.70	69.90	76.30	78.40	78.80	81.10
10	8.08	10.17	11.13	10.16	12.40	26.05	27.23	24.62	25.40	25.33	76.30	78.60	77.90	82.00	80.70
15	10.94	11.14	11.34	11.33	9.90	24.42	26.53	27.70	28.30	29.30	80.40	82.40	80.80	80.70	79.20

C.F. = Cluster Factor

not be a very effective repellent anyway.

Analysis of the number of repelled dishes is best accomplished by use of Fisher's Exact test. The large number of replications required present logistical problems for most researchers. Ten to 15 replications is a formidable amount even in small tests, and in a medium-to-large scale

screening program with a tight reading regime, data collection would be nearly impossible (by the time counting is completed for time point n, time point n + 1 may have come and gone). Unless the process could be modified for automated data collection, or destructive sampling with counting at the experimenter's leisure, the time and effort required is likely prohibitive. Alternatively, if preliminary tests are used to establish a more appropriate time interval, the data may be collected at fewer time points (or one, eliminating repeated measures altogether) allowing a larger number of replications.

Fisher's Exact test was used, and although it is a conservative test, it was the best test that was widely available in most software packages, such as SAS. Pearson's Chi squared test is unreliable when the observations are at the extreme (nearly all repelled or not repelled). The same is true of the Glimmix macro with SAS software, and the NL Mixed and GenMod procedures in SAS rely on asymptotic results, and hence are similarly affected.

Recently, Mehrotra *et al.* (2003) reported that Boschloo's Exact Unconditional test was more powerful than Fisher's Exact test, often with *p*-values one-fourth to one-half the size of those obtained by Fisher's Exact test. Unfortunately, at the time of writing no known commercially-available software package runs Boschloo's test. Mehrotra *et al.* (2003) list a webpage in their paper (http://www4.stat.ncsu.edu/~berger/tables.html) that calculates *p*-values for Fisher's Exact and Boschloo's tests. A Fortran program is also provided. Therefore, our recommendation for the number of replications may be higher than necessary, and use of Boschloo's test might reduce the number of replications required.

ACKNOWLEDGMENTS

We thank "Focus on the Workplace," Valencia Community College, Orlando, FL for providing Dr. Ems-Wilson the opportunity to participate. This publication presents the results of research only, and mention of product or firm names does not constitute an endorsement of those products to the exclusion of others.

REFERENCES

Delaplane, K.S. & J.P. La Fage 1987. Variance in feeding on equivalent wood blocks by the Formosan subterranean termite in laboratory choice tests. Sociobiology 13: 227-233.

Gleason, R.W. & P.G. Koehler 1980. Termites of the Eastern and Southeastern United States. Florida Cooperative Extension Service, Institute of Food and Agricultural Science. 192: 1-7.

Howard, R., F. Matsumura, & H.C. Coppel 1976. Trail-following pheromones of the Rhinotermitidae: approaches to their authentication and specificity. Journal of Chemical Ecology 2: 147-166.

Mehrotra, D.V., I.S.F. Chan, & R.L. Berger 2003. A cautionary note on exact unconditional inference for a difference between two independent binomial proportions. Biometrics 59: 441-450.

Oi, F.M., N.-Y. Su, P.G. Koehler, & F. Slansky 1996. Laboratory evaluation of food placement and food types on the feeding preference of *Reticulitermes virginicus* (Isoptera: Rhinotermitidae). Journal of Economic Entomology 89: 915-921.

Peterson, C.J. & J. Ems-Wilson 2003. Catnip essential oil as a barrier to subterranean termites (Isoptera: Rhinotermitidae) in the laboratory. Journal of Economic Entomology 96: 1275-1282.

Peterson, C.J., L.T. Nemetz, L.M. Jones, & J.R. Coats 2002. Behavioral activity of catnip (Lamiaceae) essential oil components to the German cockroach. Journal of Economic Entomology 95: 377-380.

SAS Institute 2001. SAS for Windows, version 8.02. SAS Institute, Cary, NC.

Sbeghen, A.C., V. Dalfovo, L.A. Serafini, & N.M. de Barros 2002. Repellence and toxicity of basil, citronella, ho-sho and rosemary oils for the control of the termite *Cryptotermes brevis* (Isoptera: Kalotermitidae). Sociobiology 40: 585-593.

Scheffrahn, R.H. & N.-Y. Su 1994. Keys to soldier and winged adult termites (Isoptera) of Florida. Florida Entomologist 77: 460-472.

Zhu, B. C. R., G. Henderson, F. Chen, H. Fei, & R. A. Laine 2001. Evaluation of vetiver oil and seven insect-active essential oils against the Formosan subterranean termite. Journal of Chemical Ecology 27: 1617-1625.