The Citrosa plant as a mosquito repellent?

Failure in field tests in upper Michigan.

BIOS 569 - Practicum in Aquatic Biology

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Field testing of the Citrosa plant

ABSTRACT. Six different repellent treatments were field-tested in the upper peninsula of Michigan for their relative effectiveness. A team of six volunteers collected mosquitoes via aspirator at sundown for 30 nights starting in May and ending in July. *Pelargonium xcitrosum* or the "Citrosa" plant was found to offer no significant repelling qualities in three different models of application used to test its reputed repellency. A burning permethrin coil also underwent preliminary tests and was shown to offer significant repellency. Comparison and analysis of the *P. xcitrosum* repellent treatments with the other models of application using *P. xcitrosum* and with a known effective repellent such as DEET and the bath oil, Skin So Soft®, offered considerable evidence that the Citrosa plant was a failure in field tests at repelling mosquitoes.

Field testing of the Citrosa plant

INTRODUCTION

Experimentation using manufactured insect repellents, especially N,N-Diethyl -M-toluamide or DEET(Buescher et. al.,1982), have offered much information and understanding of relative efficacy, overall protection, and the residue time for which they retain their repellency. Though many chemical repellents such as DEET (Curtis,1987), dimethyl phthalate (Lindstone,1990), and permethrin (Rutledge et. al.,1983) have been proven to offer significant repellent effect, both in the laboratory and in the field, some question as to their safety and possible health effects still remain open. The need for a natural and effective repellent that is safe for the consumer has gained ever increasing importance due to the parallels discovered between Anaphylaxis (Miller,1982) and a Reye-like syndrome (Heick,1980) and DEET. This concern not only has researchers searching for a biological insect repellent, but it also has them searching for more environmentally oriented products.

Interest in the Citrosa plant (*Pelargonium xcitrosum*, VanLeeni), a hybrid of the scented leaved geranium, has been expressed by researchers because of its claim to effectively repel mosquitoes by a lemony "fragrance" that mosquitoes cannot tolerate(Satran,1992). Because species of the genus *Pelargonium* tend to hybridize readily when they come in contact, morphological characteristics show that *P. xcitrosum* is most likely to be a hybrid related to *P. crispum* and *P. xlimoneum* (Bailey, 1976).

The widely accepted results on insect repellents(DEET) and other repellent products such as Skin So Soft® bath oil(Rutledge,1988) have been considerably documented, but evidence concerning the effectiveness of the Citrosa plant is extremely limited (Surgeoner and Heal,1990 as cited in Matsuda and Surgeoner,1993).

To test its efficacy, the Citrosa plant was subjected and compared to similar experimentation as DEET and Skin So Soft®, and expected to offer the levels of repellency that these exhibited under similar circumstances in the field. Three models of application of the Citrosa plant were tested and compared to results gained from similar testing with Skin So Soft® and A 95% formulation of DEET applied to the hand and forearm of the test volunteers.

MATERIALS AND METHODS

Field tests studies were conducted at the University of Notre Dame Environmental Research Center at the southern border of the Ottawa National Forest in Gogebic Co., Michigan(46° 13' N, 89° 32' E). The field tests carried out entailed evaluations of effectiveness of a number of insect repellents. This site offered

suitable habitat for mosquito production because of the numerous vernal ponds and bog lakes found on the property.

The continued biting pressure of that this site offered was compared to a similar experiment that was carried out at the same location in 1991(Staviskey, 1991) to exhibit the site's high seasonal production of mosquito populations. The mean number of strikes per person was compaired over many dates to give accurate representation of this continued production.Over a ten week period between May 17 - July 21, thirty individual experiments were carried out at dusk between 8:00 and 9:00 P.M. (Central Daylight Savings Time). Twenty volunteers between the ages of 18 and 65 participated, comprised of random groups of six, in the collection of mosquitoes on the experimental dates.

The repellents that were tested over the course of the experimental dates were Maximum Strength Muskol® liquid(95% DEET), Off® burning coil(26% permethrin), and three varied applications of the Citrosa plant(*P. xcitrosum*). Skin So Soft® bath oil(Avon Products, Inc., New York) was also tested. Its contents include mineral oil, isopropyl palmitate, diisopropyladipate, fragrance, dioctyl sodium sulfosuccinate, and benzophenone-II. The repellents were tested in a 'grouped' succession of experimental dates in an attempt to view the continuous biting pressure that the site offered over the course of the summer and the sucession of dates.

By the choice of the volunteers, the flexor region of one forearm was exposed for the two consecutive collection periods. All participants were instructed, prior to testing, to wear long sleeve shirts, long pants, effective foot and ankle cover, and a head net to impede mosquitoes from striking anywhere but the exposed region of the forearm and hand. A modified flashlight aspirator(Hausherr's Machine Works, Toms River, NJ) with a carrying frequency of 170-312 Hz and a main frequency of 350 Hz(6 aspirators) was used to suck mosquitoes landing for a blood meal into a 2.5 cm diameter by 8 cm long containment vial for identification later. The volunteers were instructed not to apply any repellents 24 hours before the test, and if they had, were asked not to participate in that night's test.

Only one repellent was tested per night . All six volunteers participated in a five minute untreated collecting period, but only five of the six received a repellent treatment for the next five minute collecting period. One subject remained untreated and out of range of those participating in the experimental group. The data collected from this volunteer was used in an attempt to display a representation of the continued biting pressure at the site by feeding mosquito populations.

The application of the DEET containing repellent and the Skin So Soft® bath oil were administered in the same fashion. The repellent was applied in spray form and then spread uniformly across the surface of the exposed forearm. An attempt to equate the volumes of the repellents used in the field was carried out in

the laboratory at a later date. This procedure yielded approximately 1 ml of each repellent.

The burning coil was ignited, allowed to smolder and emit smoke, and placed on the ground directly upwind of the experimental participants to allow them to be in a direct path of the essence of the coil.

Three different models of application were used to test the repellence of the Citrosa plant over the course of the experimental dates. First, the Citrosa plant was used as an "area" or "vapor" action repellent. The participants would either directly hold the plant by its pot without touching any part of the plant or stand directly over it. This placed them well inside of the 100 sq. ft. area that the plant is reputed to effectively repel mosquitoes(Satran, 1992). Secondly, the Citrosa plant was tested as an "applied" repellent. The experimental participants were instructed to vigorously brush the leaves of the plant over the exposed region of the forearm and then return the plant to a location outside of the range of its repellency claims so that any "area" repellent effect would be discounted. Thirdly, the Citrosa plant was ground using a mortar and pestle and rubbed uniformly across the surface of the forearm. 1 gm of leaf matter was weighed using an analytical top loader scale, ground, and then put on a piece of wax paper for transportation to the field site.

On July 12 an audio tape recording of the test site was conducted to ascertain the frequency of sound that the combined aspirators produced. This was achieved by using an electronic parabolic microphone(Dan Gibson E.P.M. P-650) and then running the tape through an ILS SIGNAL(Signal Technology Inc.) sound analysis system.

The mosquito species captured throughout the summer were *Anopheles walkeri, Mansonia perturbans, Aedes punctor, A. communis, A. abserratus, A. triseriatus, A. intrudens, A. vexans, A. provocans, A. cinereus, A. excruians,* and *A. canadensis*. The mosquitoes collected were frozen overnight and identified the following morning using The Mosquitoes of Minnesota(Barr, 1958).

All data analysis using statistical tests were conducted using SYSTAT Network Version 5.02(Wilkinson,1990). A test of the null hypothesis to ascertain whether experimental treatments offered significant repellent effect was assessed by an analysis of variance of $P \leq 0.05$. Analysis of the individual experimental nights' effectiveness were carried out by calculation of the mean densities of mosquitoes collected from the two time intervals and entered into a paired t-test statistical model to interpret significance. To ensure that a complete analysis of each repellent type was completed a one-way ANOVA was conducted to test whether there were significant differences among testing dates using the same repellent. A TUKEY analysis was also conducted to compare experimental dates using one repellent with experimental dates using another repellent. This test was performed between all combinations of the Citrosa plant, and the DEET containing repellent versus the dates of each of the other repellent grouping.

RESULTS AND DISCUSSION

Establishment of the continued biting pressure of mosquito populations on the property can be seen by comparing the data in Fig. 1. Though ranges from 30-60 strikes/person were not uncommon, differences in continued pressure and seasonal mean numbers were clearly evident for the two years' data. Figure 1 shows that while numbers collected at the beginning of 1991(season mean=20.6) were comparable to those in 1993, a late season drop-off in the mean number of mosquitoes collected may not have offered significant biting pressure for proper evaluation of the repellents tested. However, 1993's mean number of strikes/person for the season, 33.3, shows that adequate pressure existed throughout the summer, with a sharp increase in numbers collected occurring at the end of the collecting period. Fig. 2 offers an alternative view of the summer's biting pressure by showing the major species present and their relative abundance. This figure along with Table 1 displays that while *A. punctor* was the most prevalent at the end of May, its numbers decreased drastically towards early July with the emergence of *M. perturbans*. *A. communis* , though relatively small in numbers as compared to the peak emergence of *A. punctor* and *M. perturbans* , provided continued pressure during the transition of the two major species present.

Table 2 shows the results of the individual paired t-test and the mean values of both the control and experimental collecting periods for each testing date. The Skin So Soft®, DEET, and Off® burning coil treatments all showed significant repellent effect on the dates tested. Though the Burning Coil treatment was significant, substantial repellent effect was not observed. However, the mosquitoes collected during these experimental treatments had many missing legs during the identification process, a classic sign of permethrin poisoning.

Of the three different Citrosa trials tested, most offered little or no protection in terms of decreasing mean numbers(Table 2) of strikes between the control and experimental groups. However, the June 12 treatment for the Citrosa plant as an "area" repellent came up as displaying a significant repellent effect. This decrease in the biting pressure did not offer a substantial amount of protection. Fig 3b shows that there was considerable variability in the mean numbers of mosquitoes present in the two consecutive periods on each of the Citrosa testing dates. Also, since the overall numbers of the mosquitoes could not be controlled at the field site, inconsistent biting pressure and the numbers of mosquitoes collected in the previous time trial that were not "present" to strike a second time may have had an effect on the numbers present for the second trial. Figs. 4a & 4b give us an example of some individual nights within repellent treatments to offer evidence of continued biting pressure and

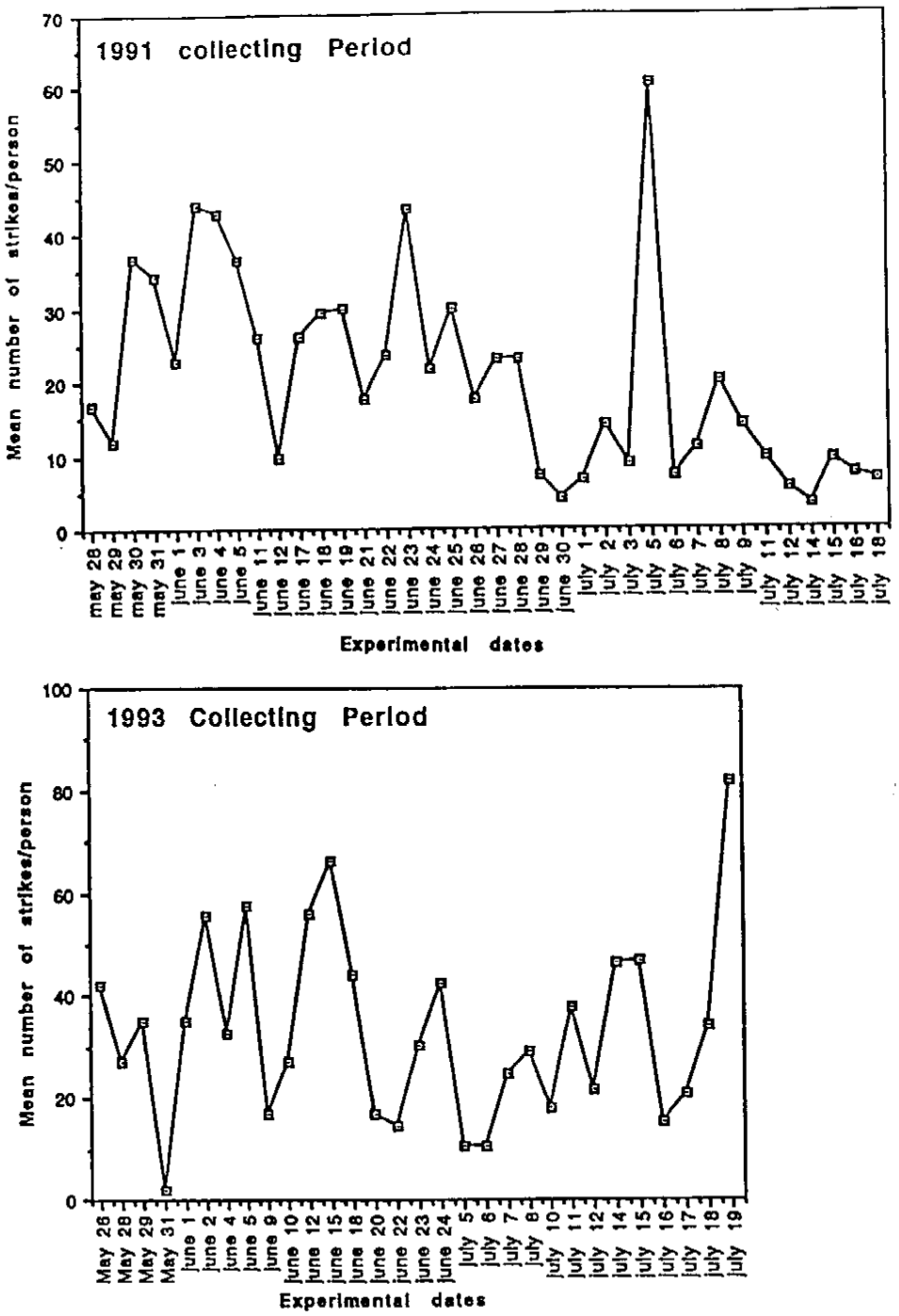

Fig. 1 Seasonal distribution of biting pressure.

Field testing of the Citrosa plant

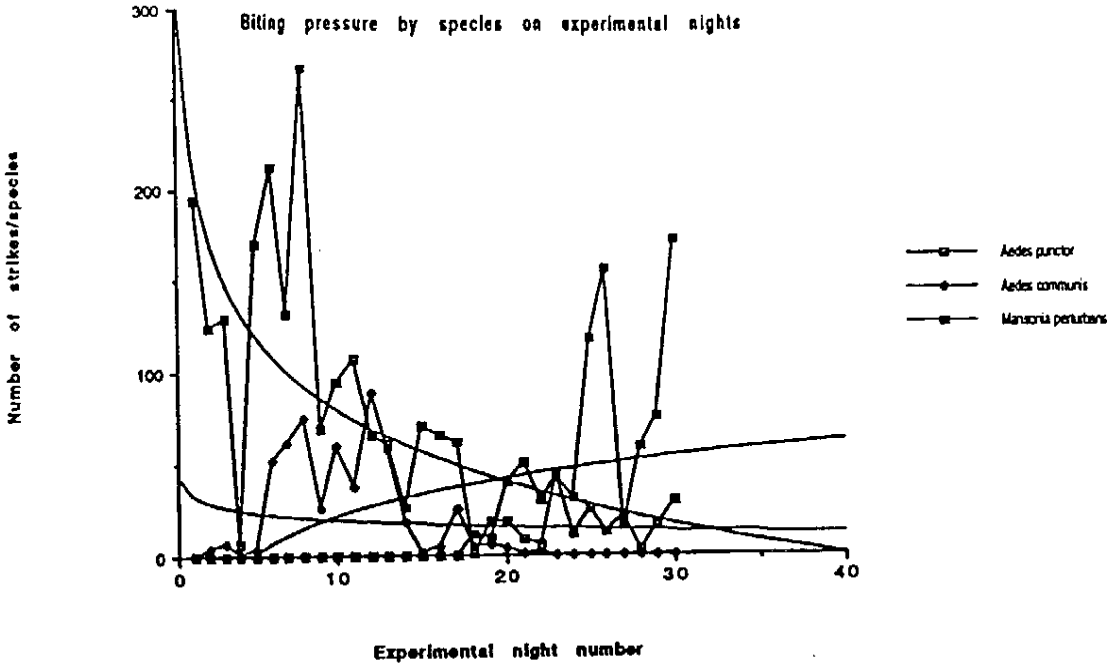

Fig. 2 Activity of major species present during 1993 collecting period.

Table 1. Numbers of major species collected by experimental date.

Experimental night	Date	A. punctor	A. communis	M. perturbans
1	26-May	194	0	0
2	28-May	124	4	0
3	29-May	129	7	0
4	31-May	7	1	0
5	1-Jun	170	4	0
6	2-Jun	211	52	0
7	4-Jun	132	62	0
8	5-Jun	266	75	0
9	9-Jun	69	26	0
10	10-Jun	95	60	0
11	12-Jun	106	38	0
12	15-Jun	66	89	0
13	16-Jun	61	58	0
14	20-Jun	26	18	0
15	22-Jun	70	2	0
16	23-Jun	66	5	0
17	24-Jun	62	25	0
18	5-Jul	1	5	12
19	6-Jul	18	6	10
20	7-Jul	18	4	40
21	8-Jul	9	1	50
22	10-Jul	6	2	30
23	11-Jul	43	0	42
24	12-Jul	12	0	31
25	14-Jul	25	0	116
26	15-Jul	13	0	155
27	16-Jul	21	0	15
28	17-Jul	3	0	59
29	18-Jul	15	0	75
30	19-Jul	29	0	171

Field testing of the Citrosa plant

Table 2. Significance displayed by repellent on its respective date.

Repellent type	Experimental dates	# of cases	Mean strikes		P Value
			Control	Experimental	
Citrosa Applied	5-Jul	4	5.0	6.8	0.060
	6-Jul	5	6.4	5.2	0.610
	7-Jul	5	12.0	13.4	0.780
	7-Jul	5	10.8	16.8	0.340
	11-Jul	5	15.8	21.2	0.124
	12-Jul	5	7.0	13.2	0.169
	14-Jul	5	26.6	20.6	0.297
	19-Jul	5	37.2	50.0	0.142
Citrosa Area	12-Jun	5	23.4	17.0	0.011
	15-Jun	5	31.2	38.0	0.223
	18-Jun	5	22.2	23.4	0.843
	20-Jun	5	9.0	10.2	0.390
	23-Jun	4	13.5	22.5	0.038
	24-Jun	5	21.6	25.2	0.712
Citrosa Ground	16-Jul	5	5.4	13.0	0.723
	18-Jul	5	17.0	16.6	0.797
Burning Coil	15-Jul	5	30.4	12.0	0.027
	17-Jul	5	8.0	2.6	0.012
DEET	4-Jun	5	30.6	0.0	0.000
	5-Jun	5	63.6	0.0	0.000
Skin So Soft	26-May	5	35.8	0.8	0.001
	28-May	5	22.6	2.0	0.005
	29-May	5	26.0	5.0	0.021
	31-May	5	1.8	0.0	0.050
	1-Jun	5	30.0	3.0	0.003
	2-Jun	5	49.2	2.8	0.008

Field testing of the Citrosa plant

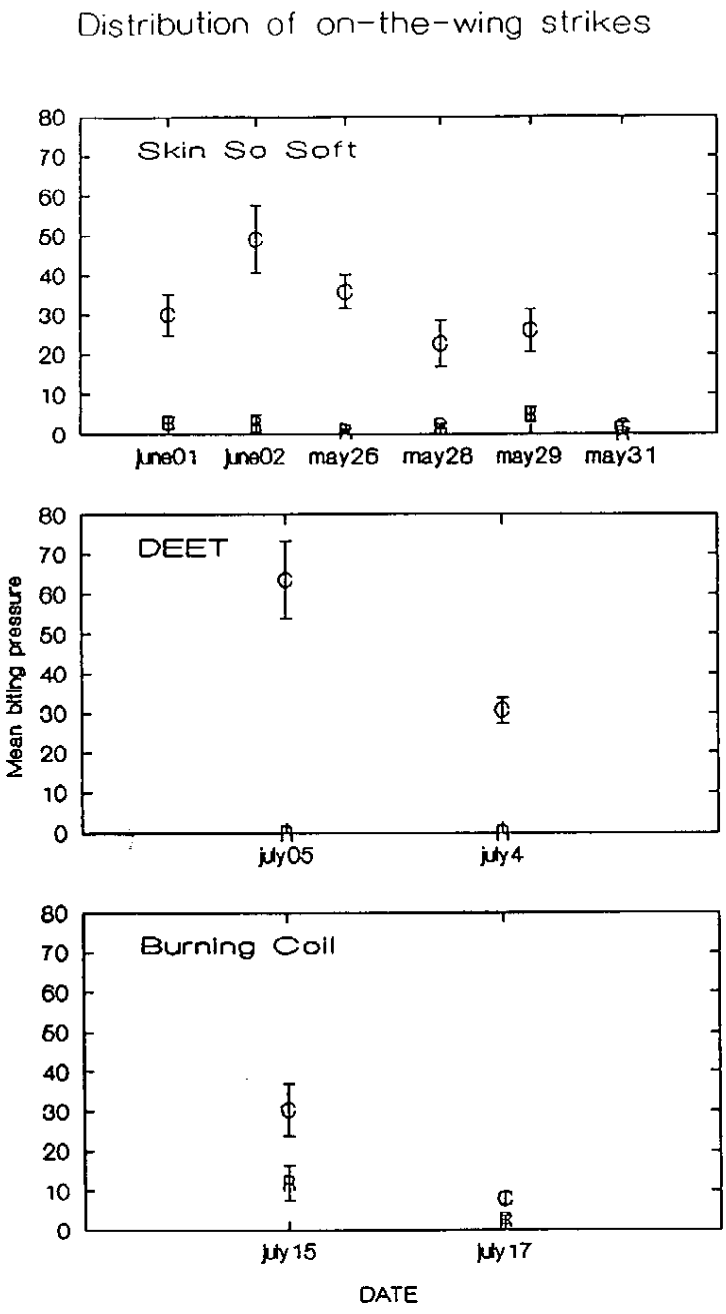

Fig. 3a Non-Citrosa datewise distribution of repellency effect

Field testing of the Citrosa plant

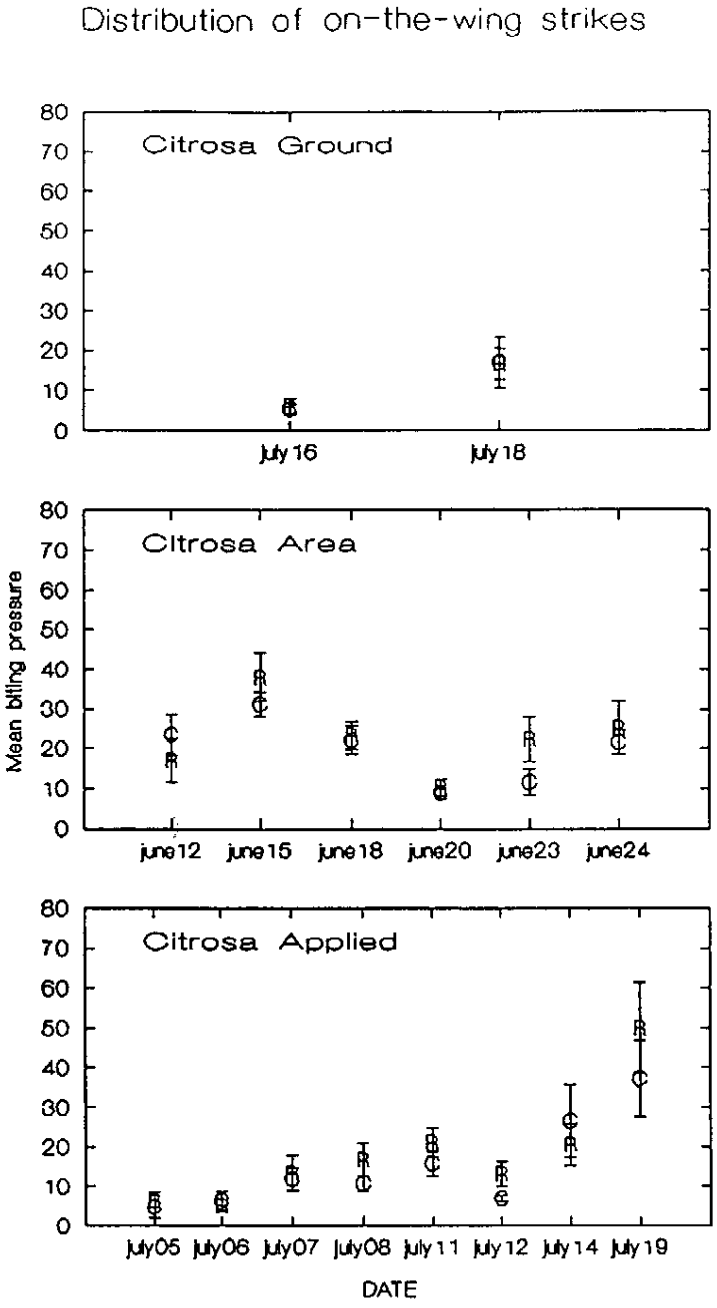

Fig. 3b Citrosa datewise distribution of repellency effect

Field testing of the Citrosa plant

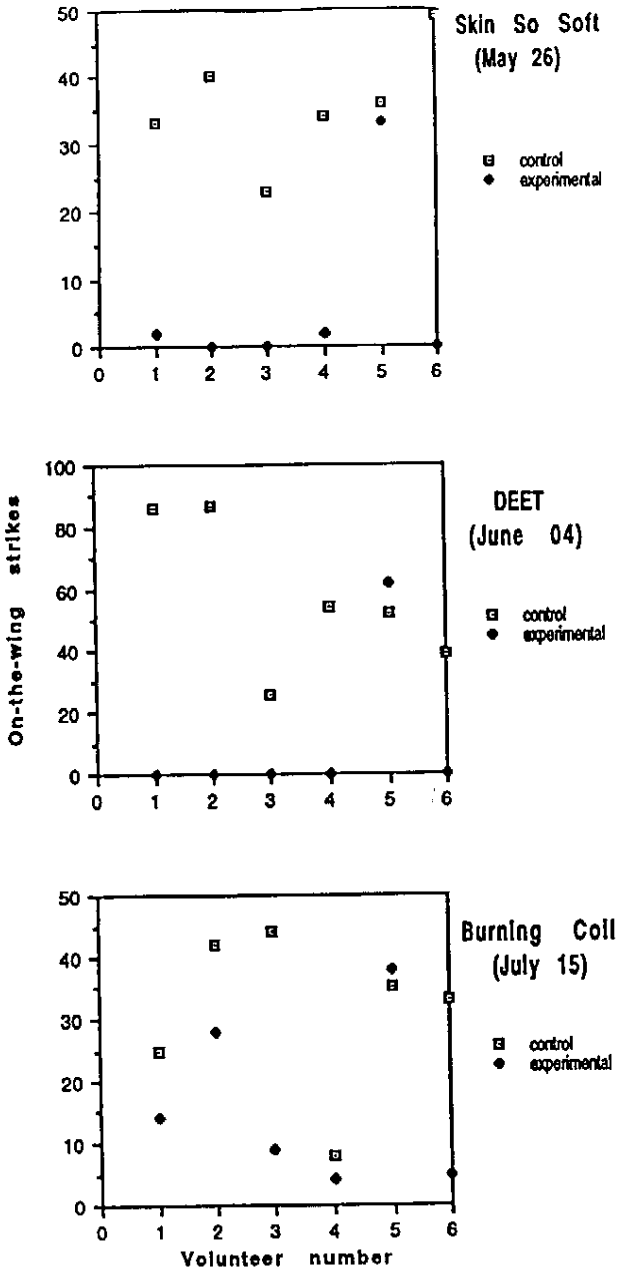

Fig. 4a Individual nights' volunteer variability with control volunteer for non-Citrosa applications.

Field testing of the Citrosa plant

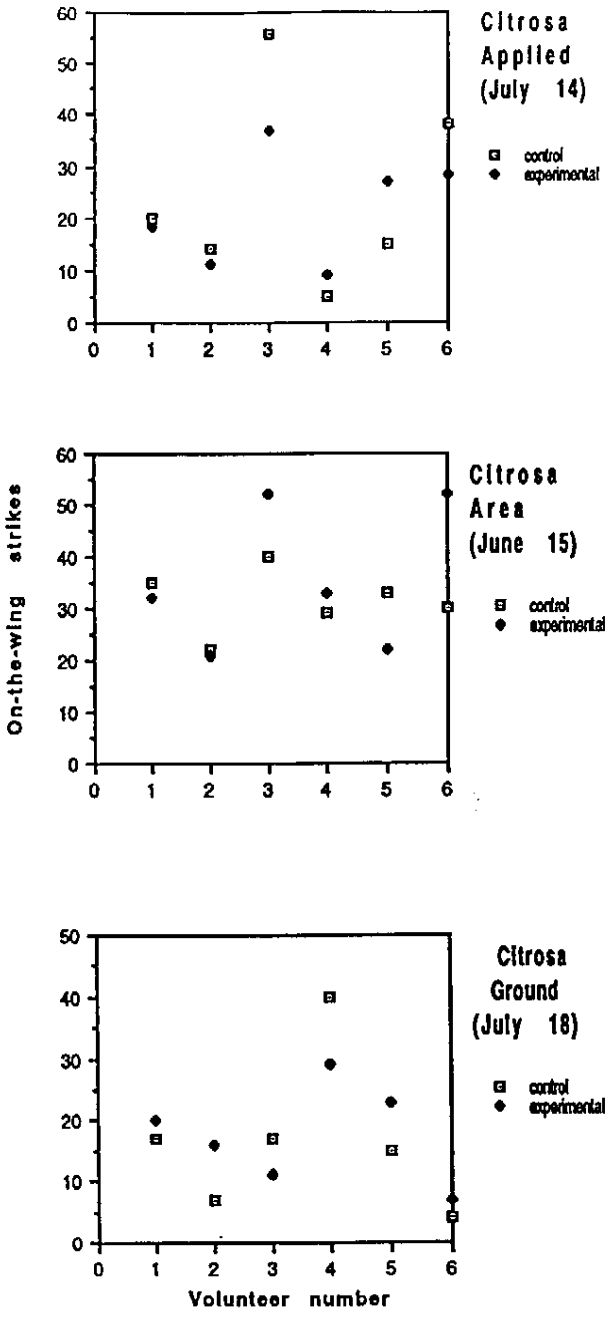

Fig. 4b Individual nights' volunteer variability with control volunteer for Citrosa applications.

variability in the numbers of mosquitoes captured by the individual volunteers. Volunteer number 5 in each treatment served as the untreated control for the experimental dates. Though continued biting pressure existed on this date, recognition of the variation exhibited by the fluctuation of feeding activity by the host seeking females over time and the varying rate of attack that existed from volunteer to volunteer may offer a possible explanation to the significant repellent activity displayed by the Citrosa treatment an June 12.

Further analysis of the variation exhibited among the experimental dates was analyzed by conducting a one-way ANOVA of the individual dates within repellent treatments. Table 3 shows that of Citrosa treatments tested, no significant differences between the dates were exhibited. The Off® burning coil test dates also showed no significant differences between the two dates that it was evaluated. Though the Skin So Soft® and DEET treatments exhibited significant differences among the dates tested within each repellent grouping, these differences can be attributed to the variability of mean numbers of mosquitoes collected on the test dates.

A pairwise comparison of the experimental dates was conducted using a TUKEY analysis to determine more accurately which dates, if any, within repellent treatments were significantly different from the other dates using the same repellent treatment. No differences were displayed in any of the Citrosa treatments or the Off® burning coil treatment(Table 4). DEET and Skin So Soft® showed differences between the dates tested. Table 4 shows that the two DEET dates were different from each other and that May 31 was significantly different from two other dates. These differences can be explained through the drastic differences in mean values showed in Table 2.

In addition to the pairwise comparison of the Citrosa treatments, A cross comparison between the dates of different repellent treatments of the Citrosa plant was carried out to determine whether the three treatments varied from each other in their repellent effect. Table 5 gives no indication of a significant difference between any of the experimental dates using different models of application of the Citrosa plant. All experimental dates were also compared to the DEET(Table 6) model to pair them with a repellent with significant repellent effect(Table 2) to give a clearer representation of the Citrosa plant and a proven product. Table 6 shows that all Citrosa experimental dates but two were significantly different from the DEET dates. These two dates' significance should be discounted on account of the pairwise(Table 4) and cross pairwise(Table 5) comparison data and the variability discussed in conjunction with Table 2. The Off® burning coil and Skin So Soft® dates exhibited significant differences, but this can be interpreted as differences in mean numbers between the dates and the variability of mosquito populations on different experimental dates.

Table 3. Significance of differences within individual repellent treatments.

Repellent type	P Value
Citrosa Applied	0.110
Citrosa Area	0.184
Citrosa Ground	0.783
Burning Coil	0.056
DEET	0.012
Skin So Soft	0.001

Table 4. Comparison of experimental dates within repellent treatments for their relative significance with respect to each other.

Repellent Type									
Citrosa Applied	Dates	5-Jul	6-Jul	7-Jul	8-Jul	11-Jul	12-Jul	14-Jul	19-Jul
	5-Jul	1.000							
	6-Jul	1.000	1.000						
	7-Jul	1.000	1.000	1.000					
	8-Jul	1.000	1.000	1.000	1.000				
	11-Jul	1.000	1.000	1.000	1.000	1.000			
	12-Jul	1.000	1.000	1.000	1.000	1.000	1.000		
	14-Jul	1.000	1.000	1.000	0.990	0.995	0.987	1.000	
	19-Jul	0.999	0.941	0.995	1.000	1.000	1.000	0.522	1.000
Citrosa Area	Dates	12-Jun	15-Jun	18-Jun	20-Jun	23-Jun	24-Jun		
	12-Jun	1.000							
	15-Jun	0.968	1.000						
	18-Jun	1.000	1.000	1.000					
	20-Jun	1.000	1.000	1.000	1.000				
	23-Jun	0.916	1.000	1.000	1.000	1.000			
	24-Jun	0.999	1.000	1.000	1.000	1.000	1.000		
Citrosa Ground	Dates	16-Jul	18-Jul						
	16-Jul	1.000							
	18-Jul	1.000	1.000						
Burning Coil	Dates	15-Jul	17-Jul						
	15-Jul	1.000							
	17-Jul	0.973	1.000						
DEET	Dates	4-Jun	6-Jun						
	4-Jun	1.000							
	5-Jun	0.001	1.000						
Skin So Soft	Dates	26-May	28-May	29-May	31-May	1-Jun	2-Jun		
	26-May	1.000							
	28-May	0.922	1.000						
	29-May	0.941	1.000	1.000					
	31-May	0.001	0.522	0.479	1.000				
	1-Jun	1.000	1.000	1.000	0.066	1.000			
	2-Jun	0.995	0.051	0.061	0.000	0.457	1.000		

Field testing of the Citrosa plant

Table 5. Cross comparison of Citrosa dates with other treatments and their relative significance.

Repellent type

		Citrosa Area					
	Dates	12-Jun	15-Jun	18-Jun	20-Jun	23-Jun	24-Jun
Citrosa	5-Jul	1.000	1.000	1.000	1.000	1.000	1.000
Applied	6-Jul	1.000	1.000	1.000	1.000	1.000	1.000
	7-Jul	1.000	1.000	1.000	1.000	1.000	1.000
	8-Jul	0.984	1.000	1.000	1.000	1.000	1.000
	11-Jul	0.992	1.000	1.000	1.000	1.000	1.000
	12-Jul	0.981	1.000	1.000	1.000	1.000	1.000
	14-Jul	1.000	0.977	1.000	1.000	0.934	1.000
	19-Jul	0.479	1.000	0.993	0.993	1.000	1.000

		Citrosa Ground	
	Dates	16-Jul	18-Jul
Citrosa	5-Jul	1.000	1.000
Applied	6-Jul	1.000	1.000
	7-Jul	1.000	1.000
	8-Jul	1.000	1.000
	11-Jul	1.000	1.000
	12-Jul	1.000	1.000
	14-Jul	1.000	1.000
	19-Jul	0.990	0.968

		Citrosa Ground	
	Dates	16-Jul	18-Jul
Citrosa	12-Jun	1.000	1.000
Area	15-Jun	1.000	1.000
	18-Jun	1.000	1.000
	20-Jun	1.000	1.000
	23-Jun	1.000	1.000
	24-Jun	1.000	1.000

Field testing of the Citrosa plant

Tabel 6. Repellent comparisons with DEET and significance.

Repellent type	Dates	DEET 4-Jun	5-Jun
Citrosa Applied	5-Jul	0.005	0.000
	6-Jul	0.009	0.000
	7-Jul	0.002	0.000
	8-Jul	0.000	0.000
	11-Jul	0.000	0.000
	12-Jul	0.000	0.000
	14-Jul	0.085	0.000
	19-Jul	0.000	0.000
Citrosa Area	12-Jun	0.099	0.000
	15-Jun	0.000	0.000
	18-Jun	0.003	0.000
	20-Jun	0.003	0.000
	23-Jun	0.000	0.000
	24-Jun	0.001	0.000
Citrosa Ground	16-Jul	0.003	0.000
	18-Jul	0.006	0.000
Burning Coil	15-Jul	0.987	0.000
	17-Jul	0.066	0.000
Skin So Soft	26-May	1.000	0.014
	28-May	0.999	0.000
	29-May	1.000	0.000
	31-May	0.013	0.000
	1-Jun	1.000	0.000
	2-Jun	0.829	0.698

Field testing of the Citrosa plant

Despite claims that the Citrosa plant is the first natural and effective means of preventing attacks by mosquitoes, field testing, the only effective way to assess a repellents commercial use, and statistical analysis point to a vastly different conclusion. Although variability in species and species number encountered during the field tests was evident, the repellency response of mosquito species' encountered during these testing dates was still significant in terms of the numbers collected. Though the fact still remains that repellency responses of one mosquito species can't be assigned to another species(Rutledge etal.,1983 as cited in Matsuda and Surgeoner, 1993), the statistical evidence available shows that the Citrosa treatments were an overall failure when compared to any species in particular.

Of the sixteen different experimental dates in which the Citrosa plant was used, demonstration of reliable repellency was not seen in any of the treatments. This failure of the repellents to offer significant protection throughout the course of the summer should not be subject to the number of statistical variables as controlled laboratory conditions because of the nature of the tests that were carried out. Surely from the evidence presented, the fluctuation of the feeding activity of the host seeking female and the varying rates of attack from human subject to human subject is clear. However, this variability cannot be controlled under any circumstance and should be discounted in an overall evaluation of the unfounded claims of the Citrosa plant. The ultimate use of a marketed product is in the field, and thus the environmental factors that the product is subject to during each trial should serve as its "control" for the variability mentioned.

However, some factors could have been examined more thoroughly to make this test more statistically standardized. The measurement of more of the environmental factors such as wind speed and direction, ambient light, and site productivity by light trapping may offer a more accurate representation of what factors influenced the mosquito species present. The human subject variability is one aspect of the experimental model that can never be accurately 'controlled' because of the nature of the agreement between the experimenter and volunteer.

Although *Pelargonium* has been cited as having repelling properties towards mosquitoes(Lewis, 1977), the scope of the experimental results point more towards the plant as merely being a scented geranium(Bailey,1949) that has received considerable attention through false claims of its repellent properties.

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