

Natural repellent field testings

**THE SUCCESS AND FAILURES OF NEW NATURAL
REPELLENTS, CITRONELLA AND NEEM OIL, AND DEET
REPELLENTS IN A NATURAL ENVIRONMENT IN
MICHIGAN/WISCONSIN.**

BIOS 569-Practicum in Aquatic Biology

Sola F. Sawyerr
245 Breen-Phillips Hall
Dr. George B. Craig
1995

Natural repellent field testings

ABSTRACT. Over a period of 56 days eight different mosquito repellents were tested on a property in upper Michigan. Repellents containing DEET, Neem oil, and citronella were used in order to compare their efficacy. At twilight, during 27 of these days, six volunteers collected mosquitoes on their forearms using these repellents in their various forms. Two citronella containing repellents were found to have a repellent effect. One of these repellents contained citronella in a spray form and one contained citronella in a cloth wipe. The third repellent that contained citronella was the Citrosa plant. It was found in some cases to have significant repellency, however overall it failed. Neem oil and Treo (a three in one sunscreen, lotion, and repellent) were found to have no significant effect. DEET in the form of a spray was found to have significant effect unlike two other products which contained DEET in a wrist band and on a tablecloth (Chase Away wristbands and tablecloths). These products had no significant effect as a repellent. The analysis of the data collected with the different repellents shows how the efficacy of the different repellents compare to each other, and how they compare to each other in their different forms.

INTRODUCTION

As early as the 1920's there have been attempts at making a good persistent formulation of a mosquito repellent (Mehr et. al.,1985). Most of the effective repellents of today have been those containing DEET, N,N-diethyl-3-methylbenzamide. DEET has become an even more popular repellent since a way of extending the protection period was found. In an experiment by Mehr et al 1985 two microcapsule formulations and one polymer formulation of DEET were found to have more than 80% protection for 12 hours, on white rabbits in a laboratory setting. Despite DEET's large effectiveness it still is known to cause problems when constantly used or used in high concentrations. Some doctors have even recommended using repellents with very small percentages of DEET (around 10%) for children (Buzz Words, 1995)

Due to problems of high DEET concentrations and the many mosquito repellents containing toxic or environmentally unsound ingredients there has been a need and an interest in natural repellents. That is, repellents that are not manufactured, but occur naturally in the environment. A couple of these repellents include citronella oil and Neem oil. Citronella oil is found in a Grass of China. There are claims that citronella oil is suppose to have a repellent effect on mosquitoes. The Citrosa plant (*Pelargonium xcitiosum*, VanLeeni) is also supposed to repel mosquitoes due to its lemony smell that mosquitoes can't stand (Satran,1992 as cited in Cummings,1993). The Citrosa plant is a cross of an African geranium and grass of China (Mitchell, 1992).

Neem oil is extracted from the seeds of *Azadirachta indica* A. Juss (Sharma, 1994). It was found that if 1 to 2% concentrations of the Neem oil is mixed with coconut oil it can act as a topical repellent of mosquitoes (Larson,1993). A 5% concentration can be burnt in a kerosene lamp for the same effect, however, neem oil only seems to work as a repellent in

Natural repellent field testings

low concentrations (Larson,1993). Neem has been proven to be safe for the environment and people and to provide complete protection for 12 hours from the anopheline, the malaria causing species of mosquitoes (Sharma, 1993)

The efficacy of citronella was tested in a spray form, plant form, and in a cloth wipe along with the other natural repellent, Neem oil as a spray. These two natural repellents were tested along side of two manufactured repellents, Treo (a three in one lotion) and DEET in it's three different forms, a spray, a wristband and a table cloth.

MATERIALS AND METHODS

The field tests of the natural and manufactured repellents were performed at the University of Notre Dame Environmental Research Center which overlaps both upper Michigan and parts of Wisconsin. At twilight, between 6:00 and 8:00 pm, a team of 6 volunteers were randomly selected each night, from a group of 20 volunteers, to participate in collecting mosquitoes while using the various mosquito repellents. These volunteers were selected from participating UNDERC students or faculty, who filled out a consent form. During the 56 days between May 5,1995 and July 24, 1995 twenty-eight testings were made.

The repellents that were used were Deep Woods Off (with 28.5% DEET), Natrapel (with citronella oil), Neem Oil in a manufacture spray, Chase Away wrist bands and table clothes, and Repello-wipes (with 8% Citronella), and the Citrosa plant. At first all of the repellents were tested in order to get an idea of which repellents were at all effective. From there all the different repellents were tested each night.

Over the summer the testings were done in three different mosquito populated testing sites on the UNDERC property. These sites were Tender Bog, Firestone Bog, and Cranberry Lake. These three location changes occurred due to variations in mosquito populations throughout the summer.

Natural repellent field testings

The methods of some of the testings were based on those of past experiments done at UNDERC, especially Cummings (1994). They involved each volunteer exposing their forearm to the mosquitoes. The volunteers were instructed to dress in clothing that did not expose other parts of their body. They all wore head nets to protect their faces and plastic gloves to protect their hands.

All six of the volunteers collected mosquitoes using an insect flashlight suction aspirator (Hausherr's Machine Works, Toms River, NJ) for five minutes without any protection on their forearm. This aspirator was used to collect all mosquitoes which landed, into pre-labeled vials. The vials were used to attach to the aspirator for easy collection of the mosquitoes. After the first five minutes of collection the vials were replaced. Then the corresponding repellents were added to the forearm of five out of six of the volunteers and the second collection was made. The 6th volunteer was used as the control to see if there was any variation in landing during the two periods. The vials for each night were frozen and identified the next day with the help of Dr. George B. Craig.

Later the data was evaluated by the same methods used by Cummings (1994) to compare their effectiveness to each other. All statistics were made using Systat for DOS (Version 6.0) with the help of Mr. Jeff Runde. A t-TEST was made for the before and after repellent application data. This test calculates probabilities, and if the probabilities are less than or equal to 0.05 then the before collections were significantly different from the after collections. A Tukey analysis was also made to compare experimental testings of one repellent to experimental testings of another repellent. This test is also based on the probabilities being less than 0.05. A graph of the mean number of landing mosquitoes per person was made in order to see the variations in mosquito populations throughout the summer. It was also compared to past experiments at UNDERC in 1991 (Stavisky) and in 1993 (Cummings) to see how the mosquito populations varied during summers in the past.

Natural repellent field testings

When topical repellents like Deep Woods Off, Natrapel, and Neem Oil were used they were sprayed on and rubbed in evenly, with a paper towel, on the forearms of the volunteers. The amount added to each person was regulated equally by the number of sprays (usually three sprays per arm). When tested, only one Citronella wipe was used for each arm of a volunteer. Due to failures of the Citrosa plant as an area repellent in past experiments (Cummings, 1993), the citrosa plant was put at the foot of each volunteer and a leaf of the plant was also rubbed all over the forearm in order to combine its area and direct repellency.

The different mosquito species collected throughout the different testings were *Aedes canadensis*, *A. cinereus*, *A. communis*, *A. excrucians*, *A. provocans*, *A. punctor*, *A. vexans*, *Anopheles walkeri*, *Mansonia perturbans*.

RESULTS AND DISCUSSION

A comparison between mosquito landing populations in 1991, 1993, and 1995 experiments is shown in the data of Fig. 1 and Fig. 2. Fig. 2 shows both the before and after mean number of strikes per person in 1995, however only the before strikes are considered in this comparison. All three years show an initially large amount of mean landings per person and then a decrease through out the summer. However the 1993 collecting period shows an increase in landing towards the end of the summer. The 1991 collecting period shows an overall decrease all the way up to the end of the summer. The 1995 collecting period showed an overall smaller mosquito population then the other previous years, that is, the controls of 1995 were quite smaller then the other years. More then half of the controls collected were less than ten mosquitoes. In the 1995 collection period there was a more drastic drop off in mean strikes per person in the late summer, during the late part of June and early parts of July. This drop in mean numbers

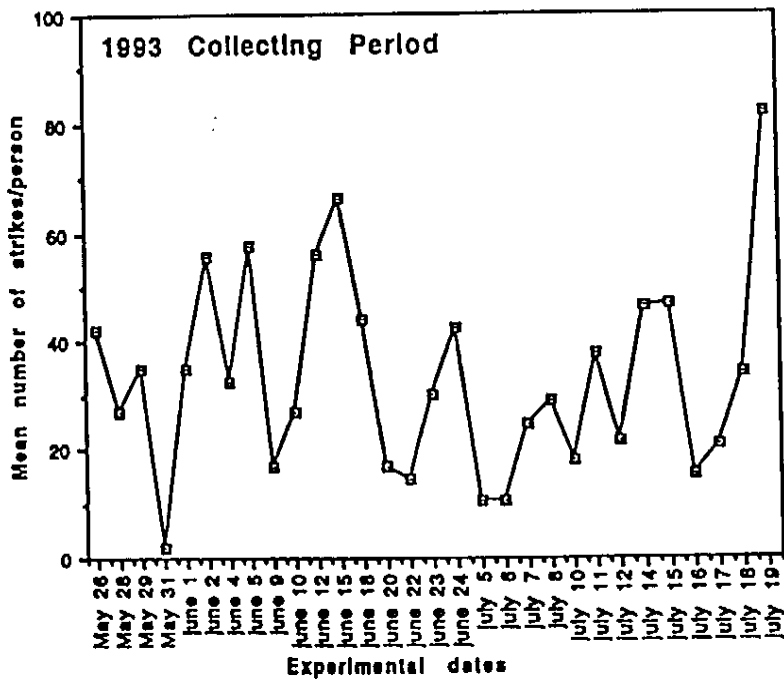
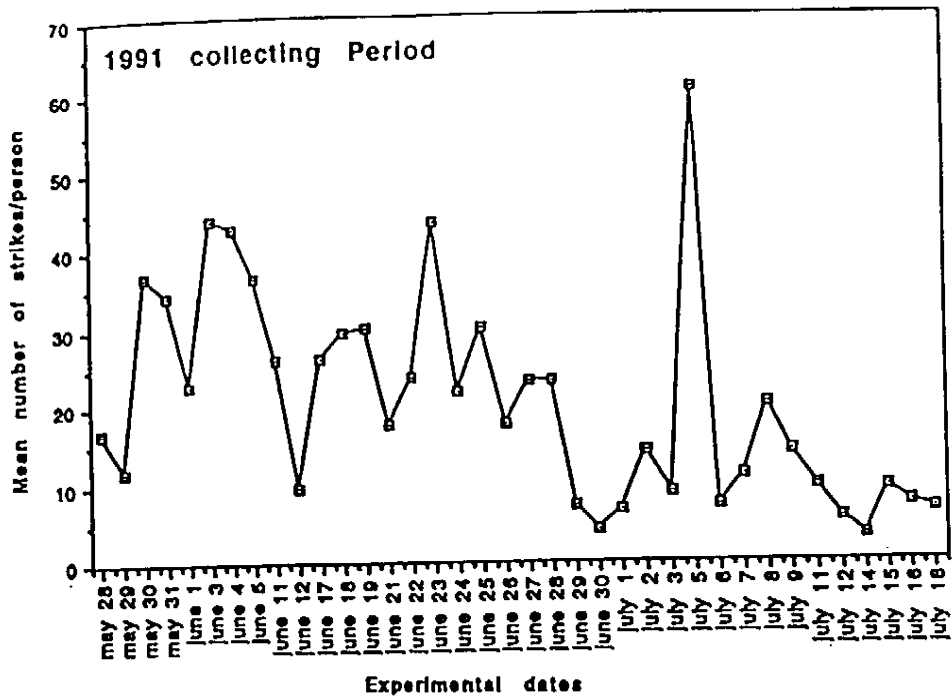
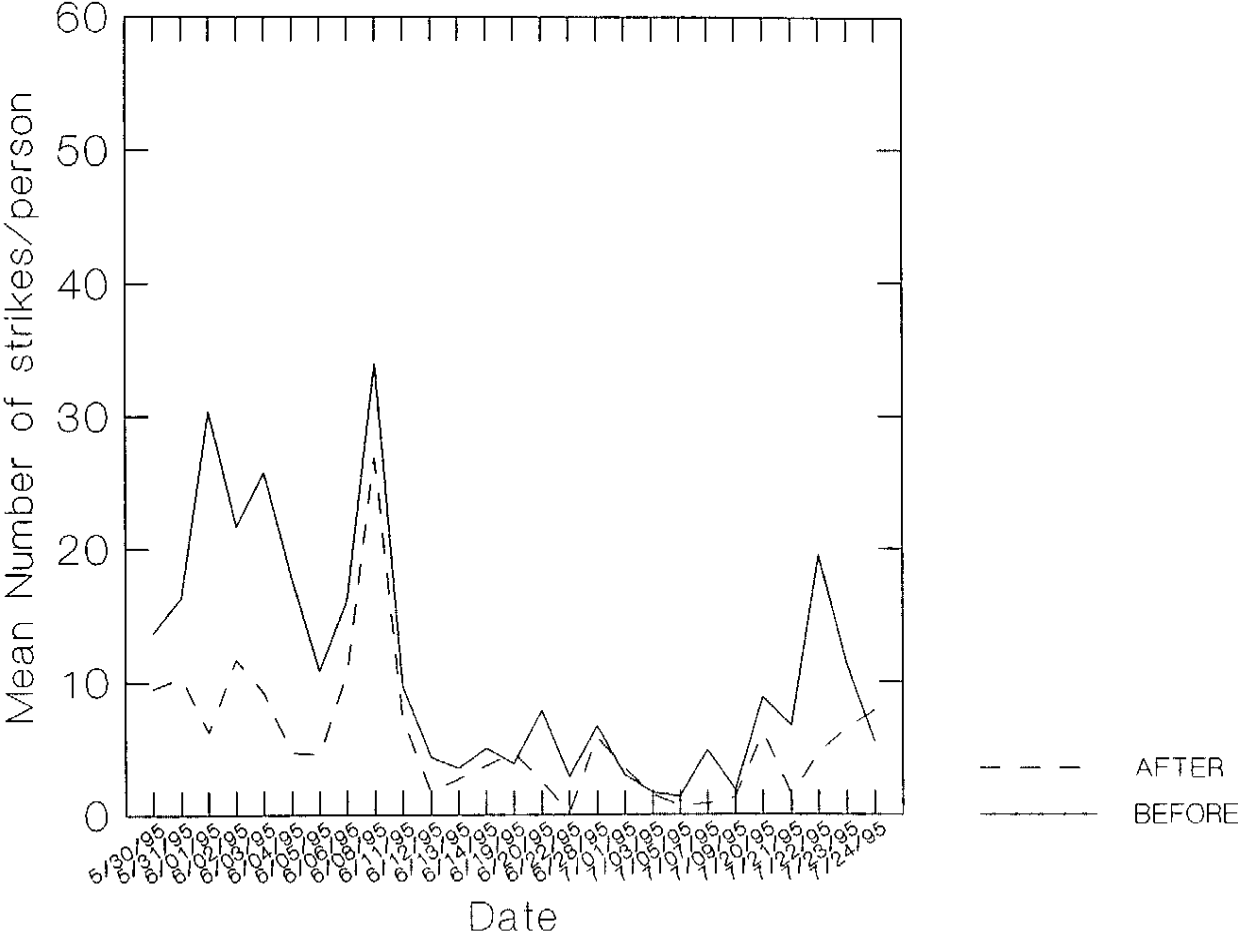


Fig. 1 Seasonal distribution of biting pressure.

Figure 2. 1995 distributions of mosquito population sizes



Natural repellent field testings

could be due to an overall period of bad weather that occurred during this time of the summer. Due to the days of bad weather, there were gaps in the data collecting. These gaps and the testing dates could have caused the discrepancy between the three different years data. However, there did seem to be a slight jump in mean numbers towards the end of the summer of 1995. This could imply a potential raise in mosquito populations towards the end of the summers on the UNDERC property. These variabilities might have caused insufficient landing populations for an accurate data analysis of some of the very low populated testings. These dates of low populated testings can be seen in Figs. 3-10. These figures show the before and after mean landing populations for all the different mosquito repellents and their different testing dates. The dates of low landing populations usually have means below 10 mean landings per person.

Table 1 shows the outcome of the paired comparison t-test and the mean values of the control and experimental collections. That is, the collections made before the repellent was added and the collections made after the repellent was added. Neem oil, citronella oil (in the Natrapel spray), OFF, Citronella wipes (Repello wipes), and the Citrosa plant all had testings in which the collection showed that they had significant repellent effects.

The Neem oil, however was tested five times and only in one of these cases was there a significant repellent effect (Table 1). Most of the experiments also did not offer protection in terms of a difference and decrease in mean control and experimental collections (Fig. 3). Citronella oil did provide significant protection in the Natrapel spray. It was tested four times and its t-test showed significant repellent effects in two of the different testing dates. All of the tests showed a significant difference between the control and experimental means except the 6/22/95 testing (Fig. 4).

OFF also showed good protection from mosquito landings. It was tested three times and the t-test showed it to have a

Figure 3. Mean mosquito landings for NEEM oil treatment.

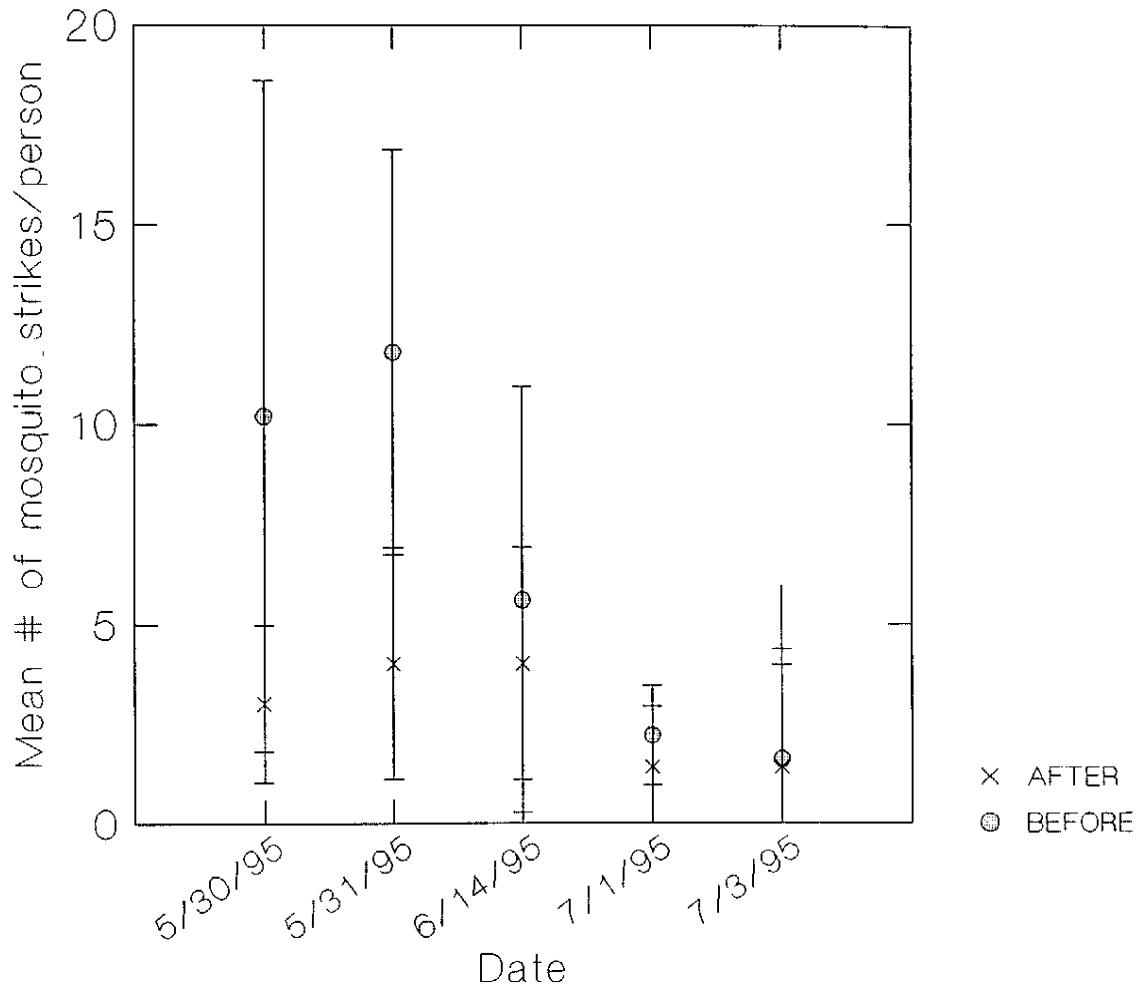


Figure 4. Mean mosquito landings for Citronella oil treatment.

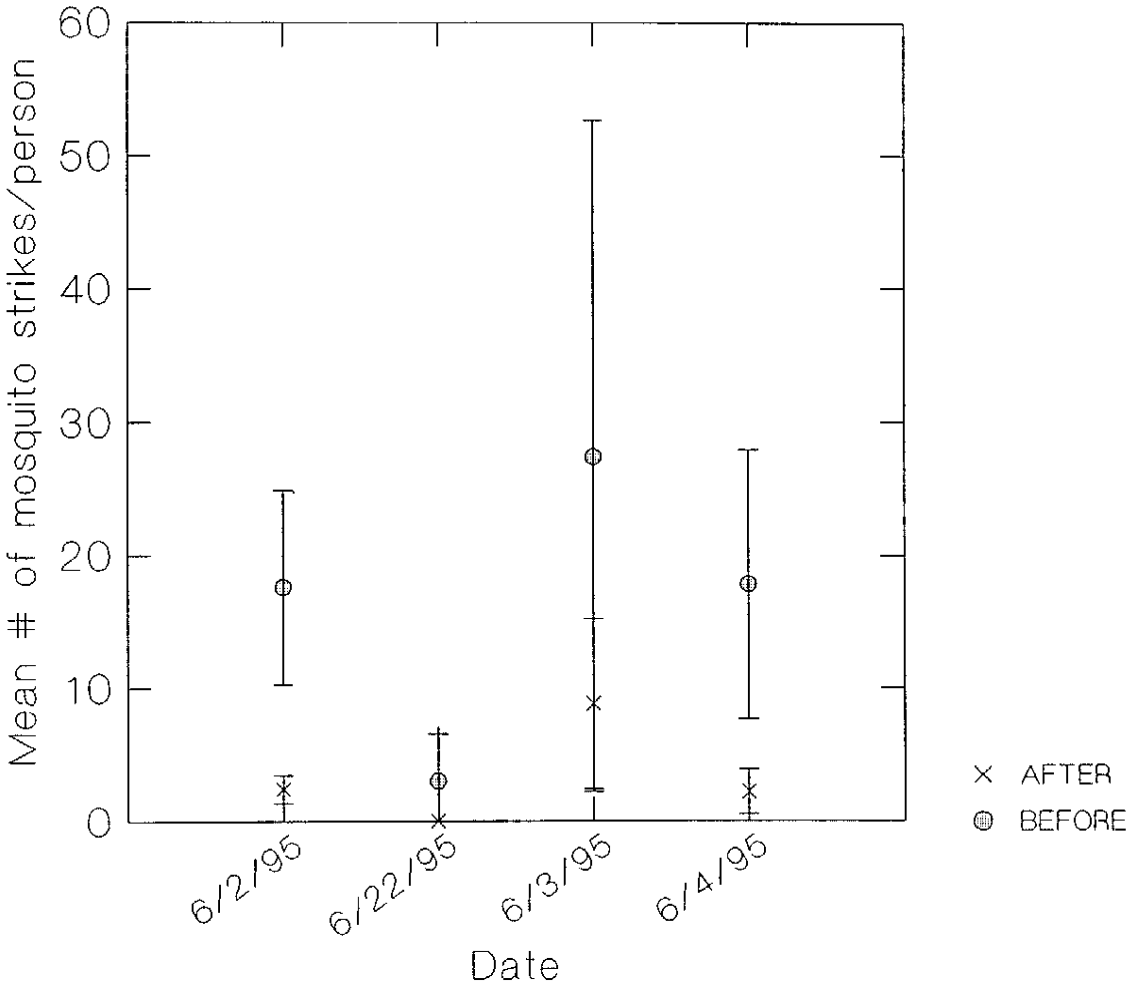


Figure 5. Mean mosquito landings for Off treatment.

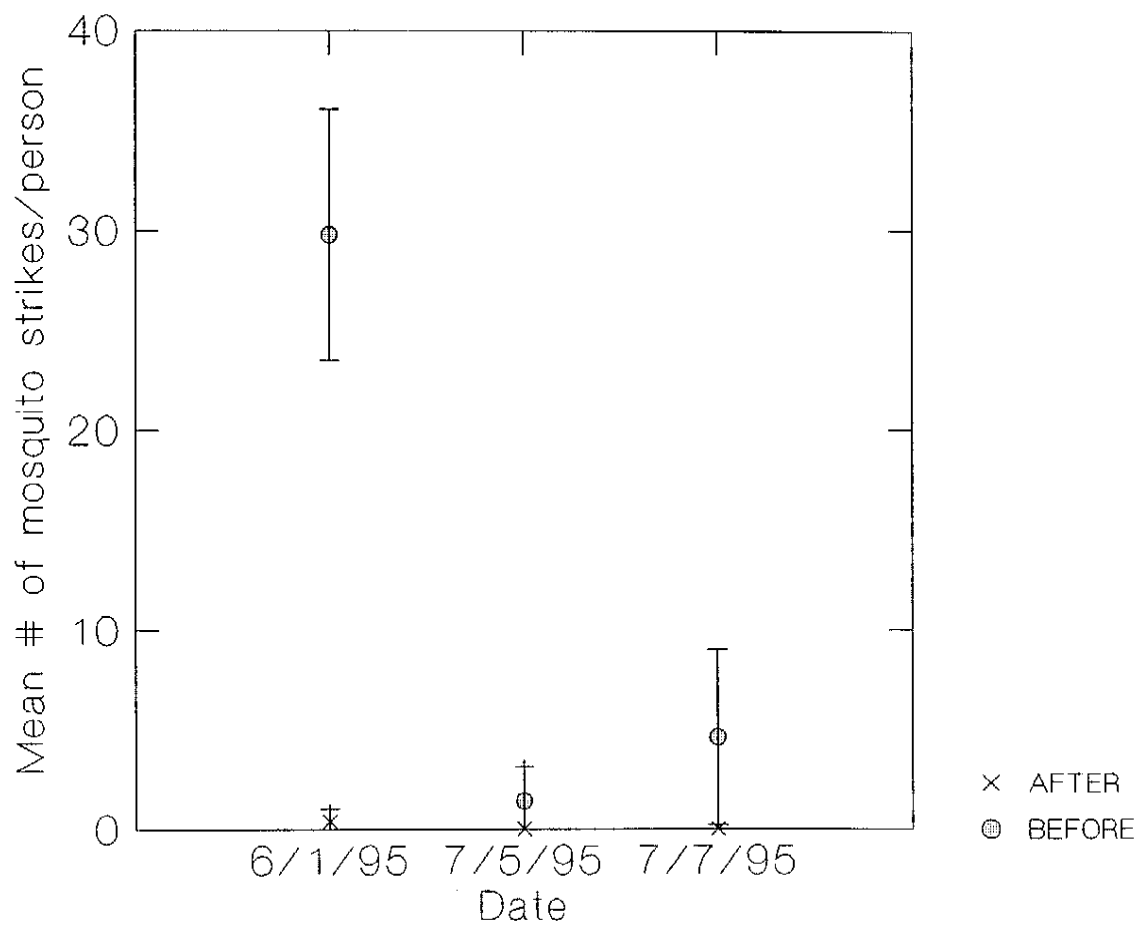


Figure 6. Mean mosquito landings for Citronella wipe treatment.

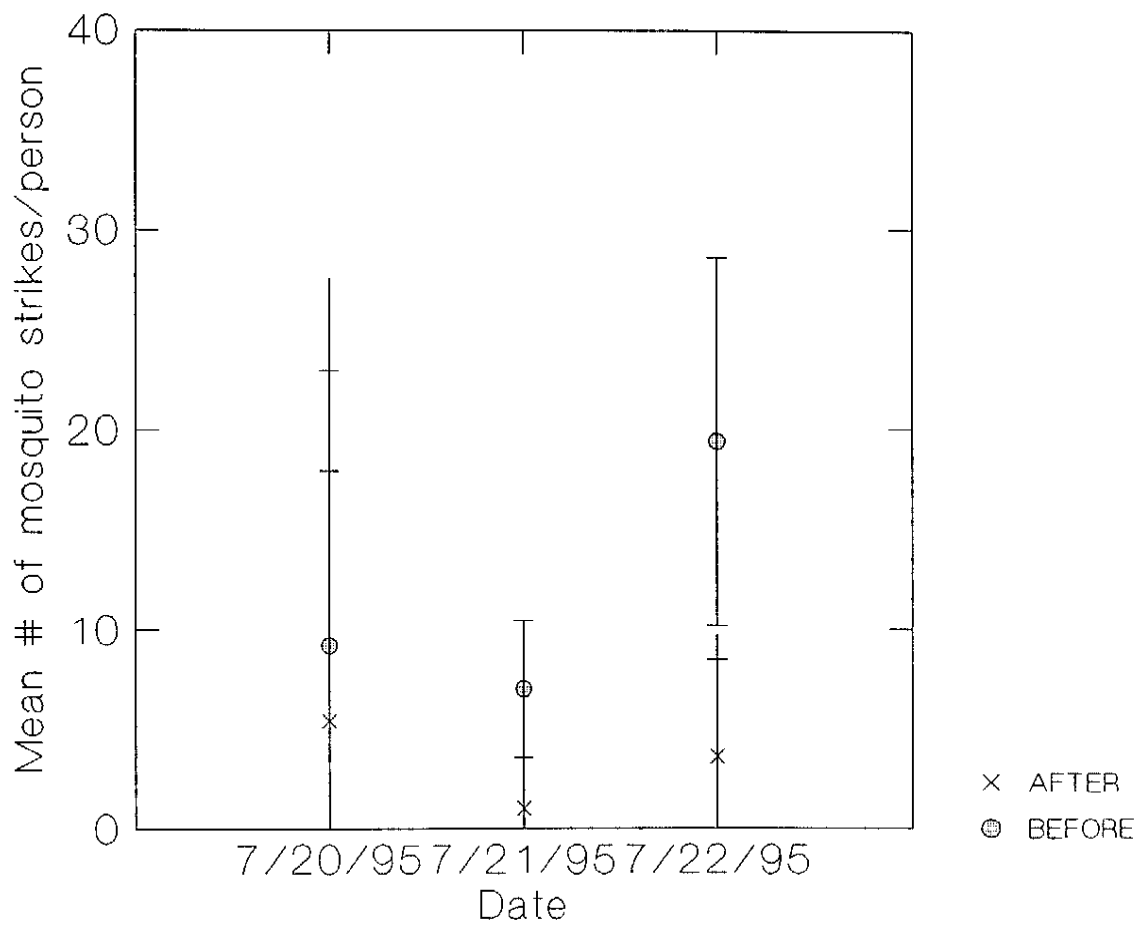


Figure 7. Mean mosquito landings for Citrosa Plant treatment.

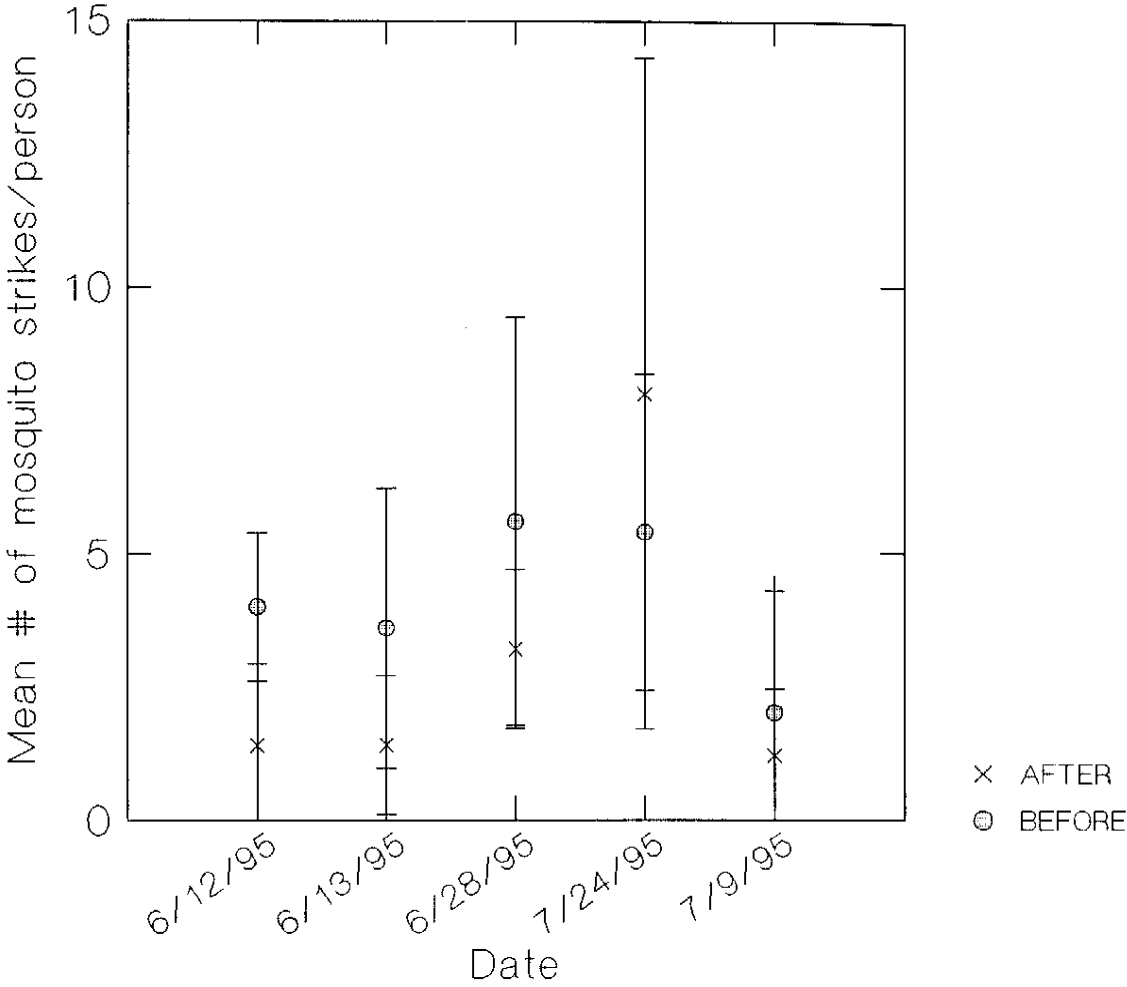


Figure 8. Mean mosquito landings for wrist band treatment.

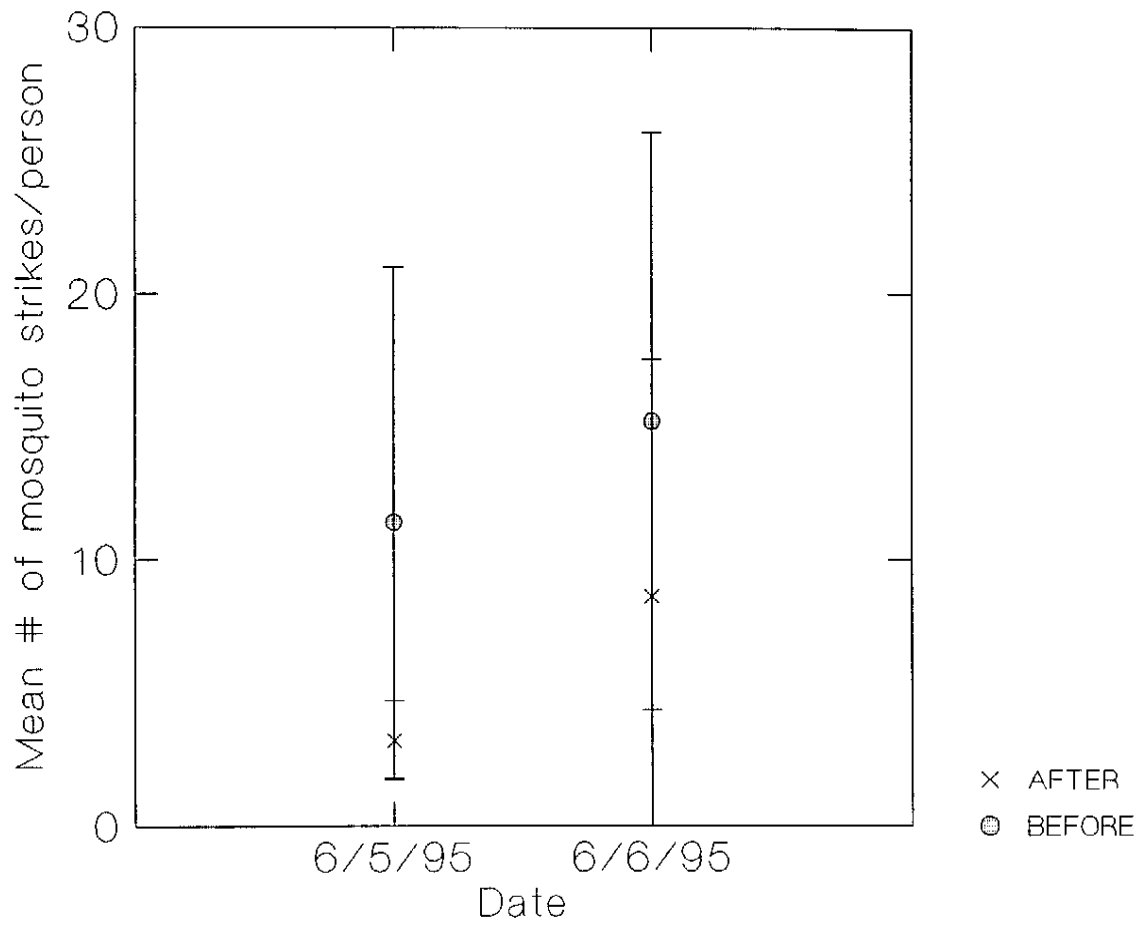


Figure 9. Mean mosquito landings for tablecloths treatment.

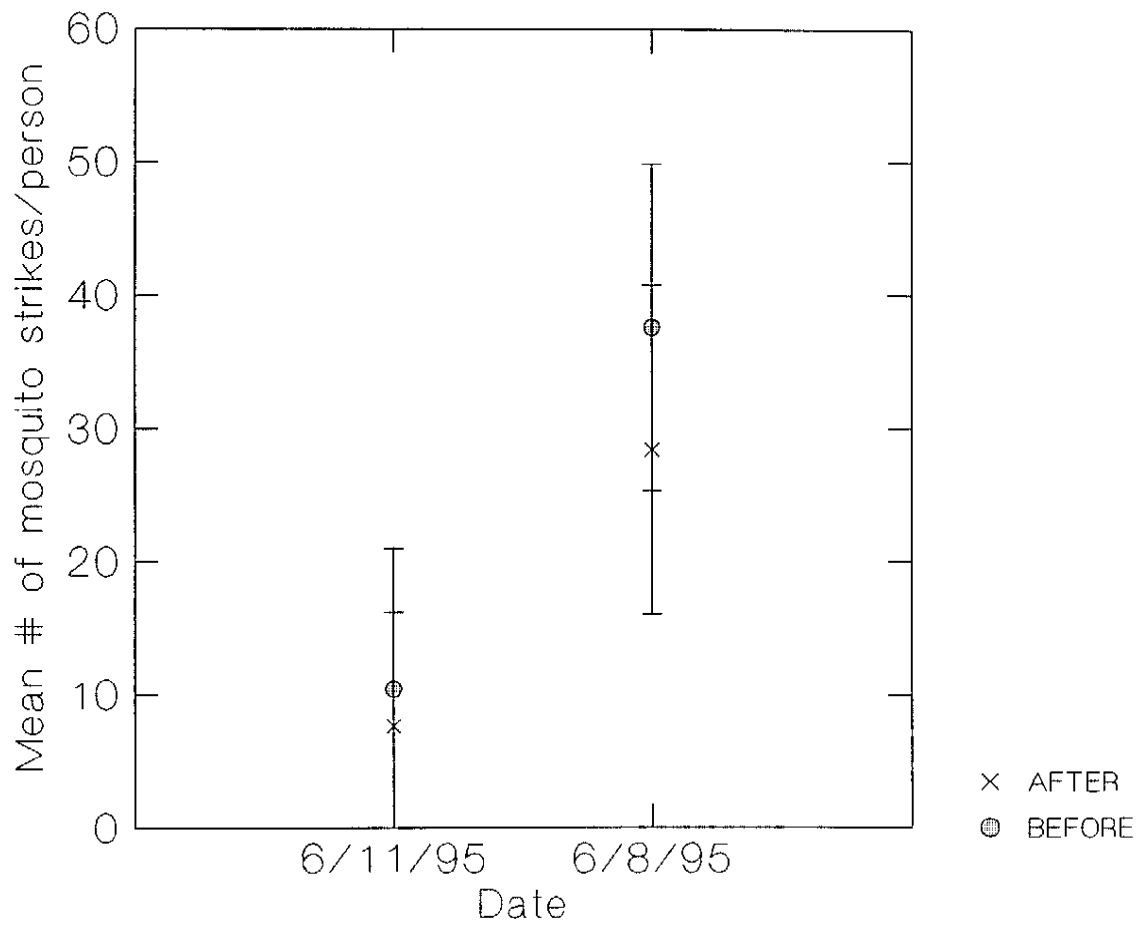
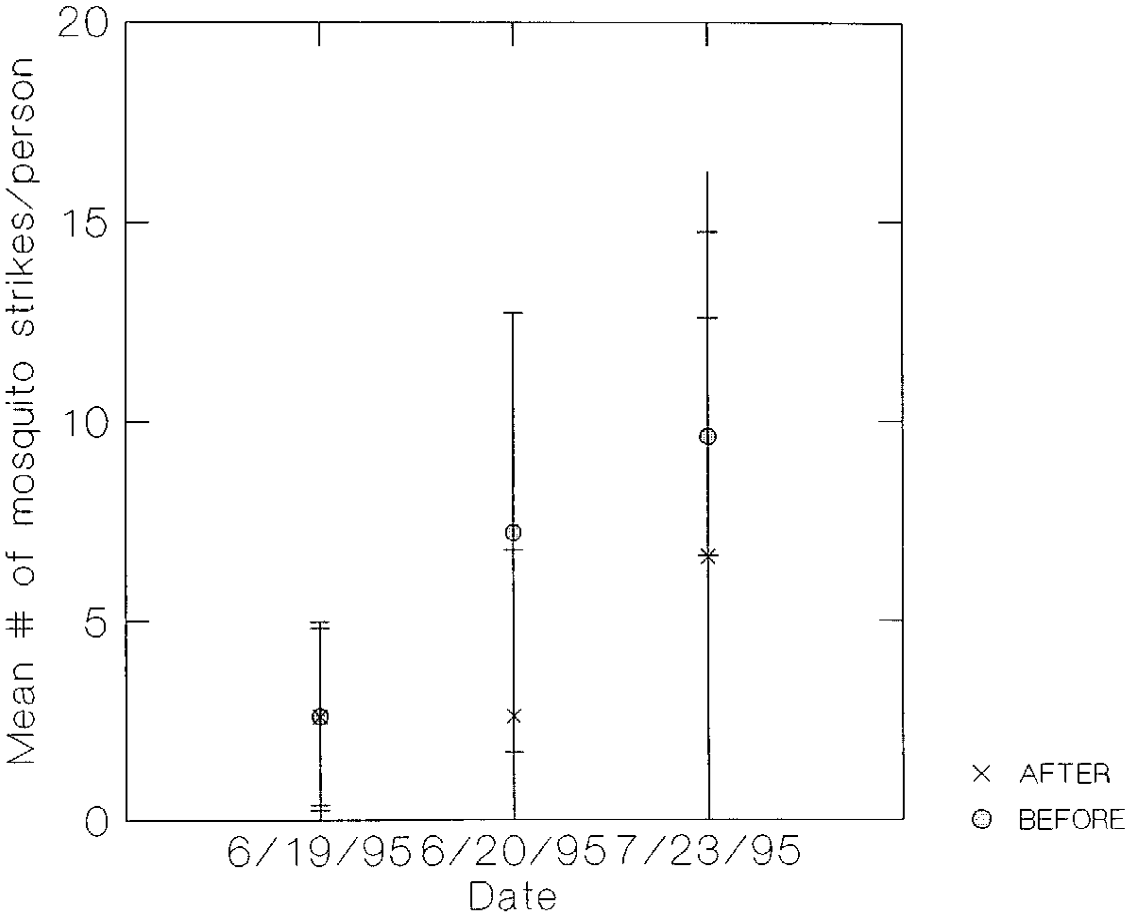


Figure 10. Mean mosquito landings for TREGO treatment.



Natural repellent field testings

Table 1: The significance of the repellents on their specific dates.

Repellent types	Dates	Control mean strikes	experimental mean strikes	P values
Neem oil	5/30	10.2	3.0	0.061
	5/31	11.8	4.0	0.023
	6/14	5.6	4.0	0.412
	7/1	2.2	1.4	0.374
	7/3	1.6	1.4	0.749
OFF	6/1	29.8	0.40	0.0002
	7/5	1.4	0	0.108
	7/7	4.6	0.0	0.056
Citronella oil	6/2	17.6	2.4	0.007
	6/3	27.4	8.8	0.109
	6/4	17.8	2.2	0.011
	6/22	3.0	0	0.095
Citrosa plnt.	6/12	4.0	1.4	0.019
	6/13	3.6	1.4	0.019
	6/28	5.6	3.2	0.118
	7/9	2.0	1.2	0.405
	7/24	5.4	8.0	0.377
Treo	6/19	2.6	2.6	1.000
	6/20	7.2	2.6	0.061
	7/23	9.6	6.6	0.308
wrist bands	6/5	11.4	3.2	0.084
	6/6	15.2	8.6	0.072
Tablecloths	6/8	37.6	28.4	0.176
	6/11	10.4	7.6	0.377
Citronella wipes	7/21	9.2	5.4	0.030
	7/22	7.0	1.0	0.030
	7/23	19.4	3.6	0.032

Natural repellent field testings

significant effect in one of the testings. This significant effect on 6/1/95 was drastic (Fig. 5). There was, however, a significant difference between the control and experimental means in all three testings. The last two testings probably did not show a significance in repellency based on the analysis due to low landing populations and not due to a lack of repellency. Despite OFF's significance in only one of its three testings, it is known from past testings at UNDERC by Stavisky, 1991 and Cummings, 1993, that it has a significant repellent effect. The two days in which it did not show significant effect based on the statistics, could be due to a mosquito population that did not allow for appropriate statistical analysis.

The Citronella wipes were tested three times. All three testings showed significant repellency based on the t-test (Table 1). This repellent offered very good protection. The testings also had significant differences between the control and experimental means (Fig. 6). The Citronella wipe showed to be a dependable repellent in all three test dates. The Citrosa plant was tested five times and on two of the dates the plant showed a significant repellent effect based on the t-test. However, the other three did not, and there was not a significant difference between the control and experimental means (Fig. 7)

Neither the DEET containing wrist band, table cloth, or Treo showed a significant repellent effect according to the t-test (Table 1). All three of these manufactured repellents failed to show much of a repellent effect in their field tests. They were all tested two times each, except for Treo which had three different testing days. Neither of the repellents showed a significant difference between the control and experimental means (Figs. 8-10)

A TUKEY analysis was made to see if there was any significant difference between different repellent testings (Table 2). By using OFF (a proven repellent) as a standard, it can be seen from the TUKEY analysis which repellents were comparative. The TUKEY analysis showed that there was a

Natural Repellent field testings

Table 2: Comparison of repellent treatments and their significance to each other.

Re- pelle nt	1	2	3	4	5	6	7	8	P values
1	1.000	0	0	0	0	0	0	0	P
2	.002	1.000	0	0	0	0	0	0	P
3	1.000	.041	1.000	0	0	0	0	0	P
4	.00003	.977	.001	1.000	0	0	0	0	P
5	.348	.996	.699	.781	1.000	0	0	0	P
6	.004	1.000	.041	1.000	.980	1.000	0	0	P
7	.765	.601	.972	.101	.998	.506	1.000	0	P
8	.656	.933	.917	.466	1.000	.861	1.000	1.000	P

Repellents: 1=citronella oil, 2=neem oil, 3=OFF, 4= Citrosa plant, 5=Tablecloths, 6=Treo, 7= citronella wipes, 8=wristsbands
P=P values

Repellents with a significant difference to each other have P values less than 0.05.

Natural repellent field testings

significant difference between OFF and three of the other repellents, Neem oil, the Citrosa plant, and Treo. This data agrees with the previous data showing that Neem oil, the Citrosa plant, and Treo did not have a significant repellent effect. This TUKEY test along with the lack of significance of three of the testings of the Citrosa plant show that the plant offers very little protection and that once again the Citrosa plant is not a success as a natural repellent in field tests as proven in Cummings, 1993.

The analysis also showed that there was not a significant difference between OFF and the Citronella spray, the Citronella wipes, the table cloth (with DEET), and the wrist bands (with DEET). This data agrees with the previous data in all cases except for the two DEET containing repellents. That is, previously it was shown that both the Citronella spray and wipes had a repellent effect, however, the other two repellents were shown to not have a significant effect. This difference in data could be due to many other unseen factors that go into the TUKEY test, because neither of the repellents show a very significant difference between control and experimental mean numbers (Fig. 7 and 8).

Many factors could have effected the data involved in this experiment, and many things could have been improved to enhance the accuracy of the analysis. An increase in experimental dates would have given more data for analysis, however, this can be limited if environmental factors like bad weather don't allow for testing. There is a definite need to only test when there is an over abundance of mosquitoes, however, this is also not a dependable source. Many different testing sites had to be used in order to find a good population of mosquitoes. A limited focus on repellent types might have improved the amount of experimental dates on one repellent. That is, it would have been better to only focus on a few repellents, in order to get enough data on them. Even though some of the above repellents were proven to be effective, further testing still needs to be done on the length of time in

Natural repellent field testings

which they will last. An experiment in which a timed testing was done on these repellents would show if they really compared to DEET, which has been proven to last up to six hours.

Despite the increase in interest in new natural repellents, the statistical evidence shows that there is a difference in how effective these natural repellents can be in different forms. There is also a potential for these repellents to not compare to DEET, which has been proven in many past studies to be an effective insect repellent. The three repellents shown to be the most effective were OFF, Citronella oil in the Natrapel spray, and Citronella oil in the Repello-wipes (Table 3). Citronella seems to be more effective in the form in which it was contained in a cloth wipe (Repello-wipe, 8% citronella oil), and it is ultimately useless when used as an area repellent in the Citrosa plant. The Citrosa plant was not effective in these field testings. Even considering the actual application of the plant to the arm of the volunteers. Neem oil as contained in its spray form also failed in these field tests. The different DEET repellents were only effective when used in their applied form of OFF. This might be explained by the potential for the chemical to lose its strength in the wrist band or table cloth form. Despite the many claims that the Citrosa plant and Neem oil are good repellents, it seems as if they are very ineffective mosquito repellents. However, the fact that repellents could vary based on their manufacturers does need to be taken into account before completely categorizing a repellent as useless.

Natural repellent field testings

Table 3: Summation of repellent efficacies

Repellent	Number of trials	Efficacy based on + scale (0 = least effective, +++ = most effective)
Neem oil	5	0
OFF	3	+++
Citronella oil	4	++
Citrosa plant	5	+
Treo	3	0
Wristband	2	0
Tablecloth	2	0
Citronella wipe	3	+++

ACKNOWLEDGMENTS

I would like to thank the Bernard J. Hank Family for providing us with the opportunity to do Environmental research on the UNDERC property. I would also like to give a sincere thanks to Dr. George B. Craig for his constant guidance and support in this research project. I would especially like to thank all the faculty and students of the University of Notre Dame and the students of the University of Wisconsin for being my enthusiastic and patient volunteers through out the summer. I would also like to thank Jeff Runde for his valuable help with all the statistical analysis in this paper. A sincere thanks is given to all these people who helped with the success of this project, without their support this project would not have been possible.

REFERENCES CITED

Cummings, R. J., G. B. Craig, Jr. 1994. The Citrosa Plant as a Mosquito Repellent? Failure in Field Trials in Upper Michigan U.N.D.E.R.C.

Florida Mosquito Control, Buzz Words. March 1995. Children's Repellents.

Larson, R. 1993. Oil drives away mosquitoes. Neem News. 1(1): 4.

Natural repellent field testings

Mehr, Z.A., L.C. Rutledge, E.L. Morales, V.E. Meixsell and D.W. Korte. 1985. Laboratory evaluation of controlled-release insect repellent formulations. *J. Am. Mosq. Control Assoc.* 1(2): 143-147.

Mitchell, Lee. Summer 1992. Mythical Mosquito Control. *Wing Beats* 18-20.

Satran, B. 1992. Citrosa Villas County. *Villas County (Wisconsin) News-Review*.

Sharma, V.P., M.A. Ansari, and R.K. Razdan. 1993. Mosquito repellent action of neem (*Azadirachta Indica*) oil. *J. Am. Mosq. Control Assoc.* 9(3): 359-360.

Sharma, V.P. 1994. Personal protection from mosquitoes. *J. Med Entomol.* 31(3): 505-507.

Stavisky, J. 1991. The effectiveness of mosquito repellents. U.N.D.E.R.C.