THE EFFECT OF BASAL TREEHOLE CLOSURE ON SUPPRESSION OF *AEDES TRISERIATUS* (DIPTERA: CULICIDAE)[1]

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ABSTRACT. Closure of basal treeholes to prevent breeding by *Aedes triseriatus* (Say) gave only limited success in control of this mosquito in southwestern Wisconsin. At 4 test sites, percentage reductions as measured by egg deposition in ovitraps were 91.1, 70.0, 65.9 and 0 when seasonal means were compared with those of similar sites in which treeholes were left undisturbed. One factor that appeared important in reducing the effectiveness of this method was infiltration of treated areas by *A. triseriatus* from surrounding woodlands. Eggs deposited in an ovitrap hung on an isolated tree between 2 woodlots otherwise separated by 95 yards of grassy terrain demonstrated that *A. triseriatus* ventured readily into this open zone. Other factors that appear to be important include arboreal treeholes unless they can be found and eliminated, discarded tires unless they are removed, and increased rainfall.

The results of laboratory transmission experiments (Watts et al. 1972, 1973a, b) and previously existing evidence indicate that *Aedes triseriatus* (Say) is the major vector of LaCrosse virus (California encephalitis group) in the upper Mississippi Valley. *A. triseriatus* under natural conditions breeds almost exclusively in water-containing treeholes and this suggested that closure of the treeholes might be an effective method of suppressing populations of this mosquito in localities where the human population is under risk of infection with LaCrosse virus.

In southwestern Wisconsin where LaCrosse virus is endemic most treeholes are rot-holes according to the classification of Kitching (1971) and are generally caused by extrinsic factors. These factors have been discussed by Hanson and Hanson (1970). Our studies were initiated in 1971.

MATERIALS AND METHODS

Four sites were selected in southwestern Wisconsin and at each site an attempt was made to find and fill all basal treeholes. These sites were coded T for treatment, and the main considerations in their selection were presence of treeholes and some degree of isolation from surrounding wooded areas.

For each treated area a 2nd site was selected to serve as a control. Control areas were coded C and their treeholes left undisturbed. Each control area was selected on the basis of proximity to and similarity to its corresponding treated area in size, degree and direction of slope, and condition of vegetation. Treated and control areas that were paired with each other were identified by the same numbers, i.e., C1 was the control area for treated area T1.

All of the areas studied except C3 and T3 were located in the Spring Coulee area of Vernon County about 10 miles south of LaCrosse, Wisconsin. This is an unglaciated area in which hardwood forests persist on the steep hillsides while most of the ridge tops and valleys have been cleared for farming.

Treatment area 1 (T1) consisted of 20 A on a north-facing slope (Fig. 1a). Powerline clearings, each 30 to 40 yd wide, formed the east and west boundaries. In 1971 when this experiment was conducted there were shrubs and/or small trees at certain points under both powerlines. Cleared farm land formed the north and south boundaries except for a connection 60 yd wide at the top of the hill with woods (Area S) extending up from the opposite side. Sixty-seven basal treeholes were found and closed in T1. Control

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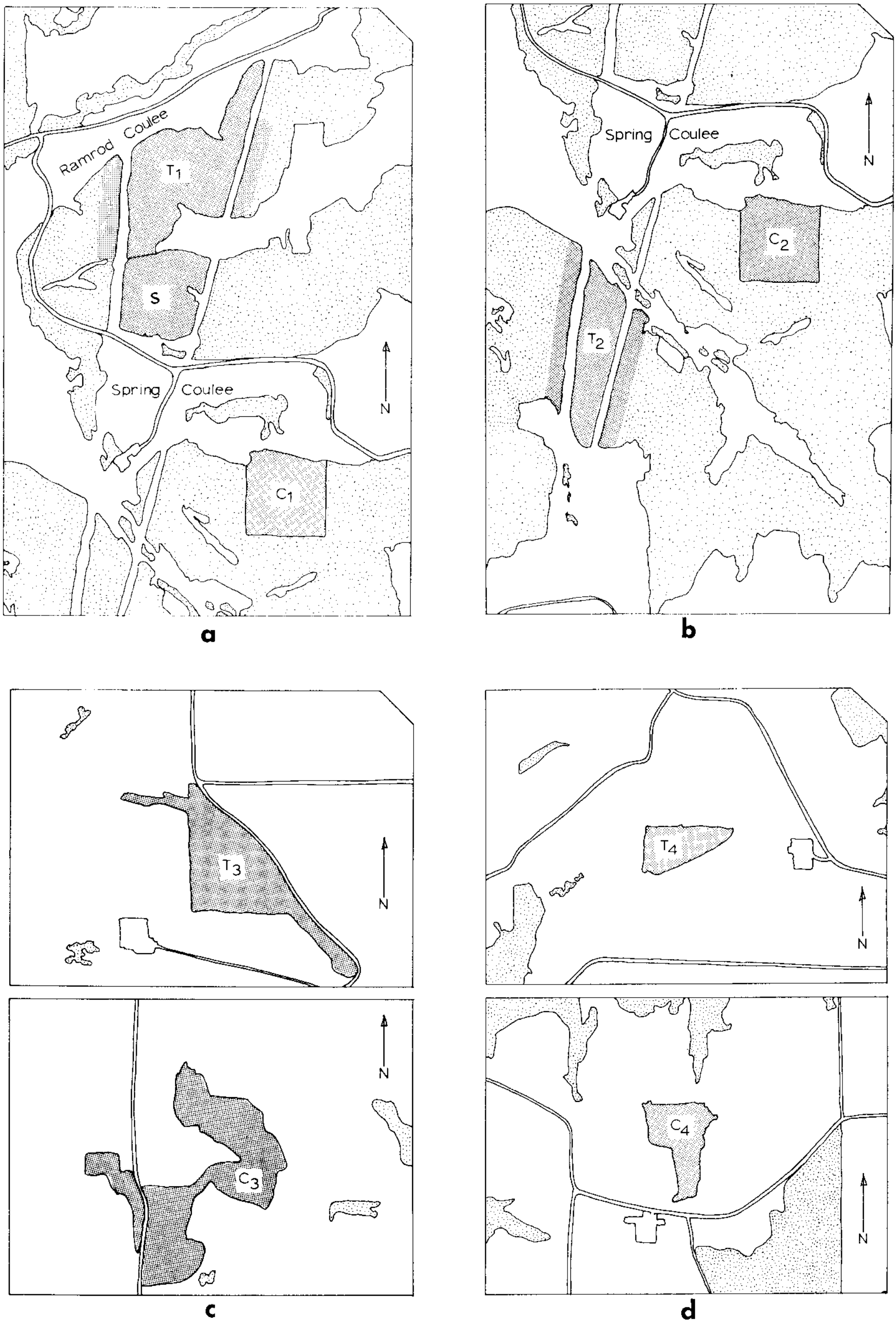

Fig. 1. Treatment (T) and control (c) areas monitored for *Aedes triseriatus* oviposition activity in southwestern Wisconsin, 1971–1973.

area 1 (C1), also on a north-facing slope (Fig. 1a), was a 15 A site that was part of a more extensive wooded hillside.

Treatment area 2 (T2) (Fig. 1b) was used in 1972 and consisted of 10 A on a north-facing slope. It was somewhat better isolated than T1 in that there was no direct connection with other wooded areas. It was, however, within 30 to 40 yd of adjacent forest. Twenty active basal treeholes were found and closed. Control area 2 (C2) was the same as the area that had been designated as C1 in 1971.

Treatment area 3 (T3) (Fig. 1c) was located in northern Green County 3 miles southwest of Belleville, Wisconsin. It consisted of 15 A on an eastern slope and was separated from other wooded areas by 440 yd of open terrain. In this area 36 active basal treeholes were found and closed. Control area 3 (C3) (Fig. 1c) was located in southern Dane County 2 miles southwest of Paoli, Wisconsin. It is a well-defined woodlot of 15 A with a mostly eastern slope.

Treatment area 4 (T4) (Fig. 1d)was on a plateau and was a relatively flat 8 A woodlot separated from the nearest woods by 360 yd of open farmland on which were interspersed a few trees. This area contained 5 active basal treeholes which were closed. Control area 4 (C4) was a flat area of 10 A on the same plateau.

In a separate experiment designed in part to determine whether *A. triseriatus* moves into broad open areas, ovitraps were placed in 2 untreated wood areas approximately 95 yd apart. Five traps were placed in 1 of the areas, a 5 A wooded hill (Kaeser Farm), 2 in the other woodlot, and 1 trap was located on an isolated tree midway between the 2 areas.

At all sites treeholes were found by first dividing each area into small sections using white cord to demarcate narrow corridors. The width of these sections depended on the number of searchers available. The surveys were conducted each spring after ice had melted in the holes and before new vegetation appeared on the forest floor. Each tree was inspected up to head height. Water-filled treeholes were marked with red flagging tape and dry holes were marked for continued surveillance. All waterholding treeholes were then drained and packed with rocks and soil. A concrete mortar cap over dirt filling was used in 1971, but this was not considered necessary when working in small areas where treeholes could be checked regularly, and the procedure was abandoned.

To monitor oviposition, ovitraps (Loor and DeFoliart 1969) were constructed from soda cans with 1 end removed. They were painted a tan enamel to make them neutral in nature and uniform. The black cotton liner for the inside of each can extended over the top, thus permitting oviposition only on 1 side of the liner. The liner was changed weekly at each site and the used liner was returned to the laboratory in a marked plastic bag.

Trap density was 1 per acre which is half the number of active treeholes per acre frequently found in southwestern Wisconsin. Traps were placed in a pattern estimated to best represent the variability of an area, but never within 200 ft of each other.

In the laboratory liners were flattened and partially dried in preparation for counting the eggs. To facilitate accuracy of the counts under a binocular microscope a counting board was constructed which divided the liner into sections equal to the width of the field of view at 7×. Occasional recounts of the eggs on a liner confirmed the accuracy of the method. After the count was recorded the liner was returned to the original plastic bag for storage.

To determine whether the majority of eggs in each area were *A. triseriatus* or *A. hendersoni* Cockerell, during 1971 eggs on a sampling of liners were hatched, the larvae reared to 4th instar and keyed according to the 5 main characters used by Truman and Craig (1968). Subsequently, eggs from collections were hatched and the larvae identified only on a spot check basis.

To determine if a significant difference occurred between study areas, Student's t test was applied to the data, both for each week and for each season as a whole. Because of the unclosed treeholes in the control areas and the presence of ovitraps in the same density as in treated areas, it was necessary to transform the data for the control areas to make a fair comparison possible (M. G. Karandinos, University of Wisconsin, personal communication). To do this a factor was computed that accounted for the number of closed treeholes in the treatment areas. The t test was also used to test the significance within areas of differences in collections made on one side versus another, or the importance of edge or physical factors such as presence of rubbish.

RESULTS

During the years of this study egg deposition by *A. triseriatus* began between the 2nd week and the 4th week of June and continued into early October, a period of approximately 15 wk. Seasonal curves revealed a minor peak or peaks in July, and the major peak or peaks in mid to late August or early September. Egg deposition decreased more sharply at the end of the season than the rate at which it increased at the beginning of the season. Exceptions to this pattern were Area S, a southern exposure which showed major peaks in July and minor ones in August, and area T4, a relatively flat area which showed major peaks in the 2nd and 4th weeks of July and a substantial peak in late August.

Identification of mosquito larvae reared from ovitrap-collected eggs showed that 2 species, *Aedes triseriatus* and *A. hendersoni,* utilized the ovitrap, but that the proportion of *A. hendersoni* and its hybrids was not great enough to justify hatching and identifying all eggs collected. Of 624 larvae identified from area C1, only 14 (2.2%) were considered to be *A. hendersoni* and hybrids (Grimstad et al. 1974). Spot checks of other areas substantiated this observation, though 1 area, T3, displayed a somewhat higher propertion of *A. hendersoni.*

1971: AREAS C1 AND T1. Concurrent with each peak in C1 was a subtle increase in T1 (Fig. 2a). The treatment and control areas were statistically different for the season as a whole (Table 1) and for weekly comparisons except during the 3rd and 4th weeks of June. Overall oviposition reduction was 91.1% if seasonal means are compared, 89.4% if seasonal sums are compared. Traps in T1 collected an average of 799 eggs during the season.

The trap in T1 that collected the greatest number of eggs was located in the upper-west corner of the area near the wooded connection to untreated south-facing woods. In this south-facing plot (Area S) oviposition peaked very early in the season. It began 1 week earlier than in the control area, C1, and during

Table 1. Comparison of yearly means and total eggs oviposited by *Aedes triseriatus* in all study areas, 1971–1973.

		Eggs					Percent reduction	
Year	Areas	T Mean	C Mean	T Sum	C Sum	t Value[a]	Mean	Sum
1971	C1T1	799.6	9027.6	14393	135419	11.273**	91.1	89.4
1972	C2T2	2723.6	9080.8	54471	136212	9.739**	70.0	60.0
1973	C3T3	2502.5	7349.2	37538	110238	5.374**	65.9	65.9
1973	C4T4	4373.6	4117.2	34989	41172	0.316[ns]	−6.2	15.0
All	All	....	....	141391	432041	....	....	66.6

[a] **=resulting means significantly different at the 1% level: ns=resulting means not significantly different at the 5% level.

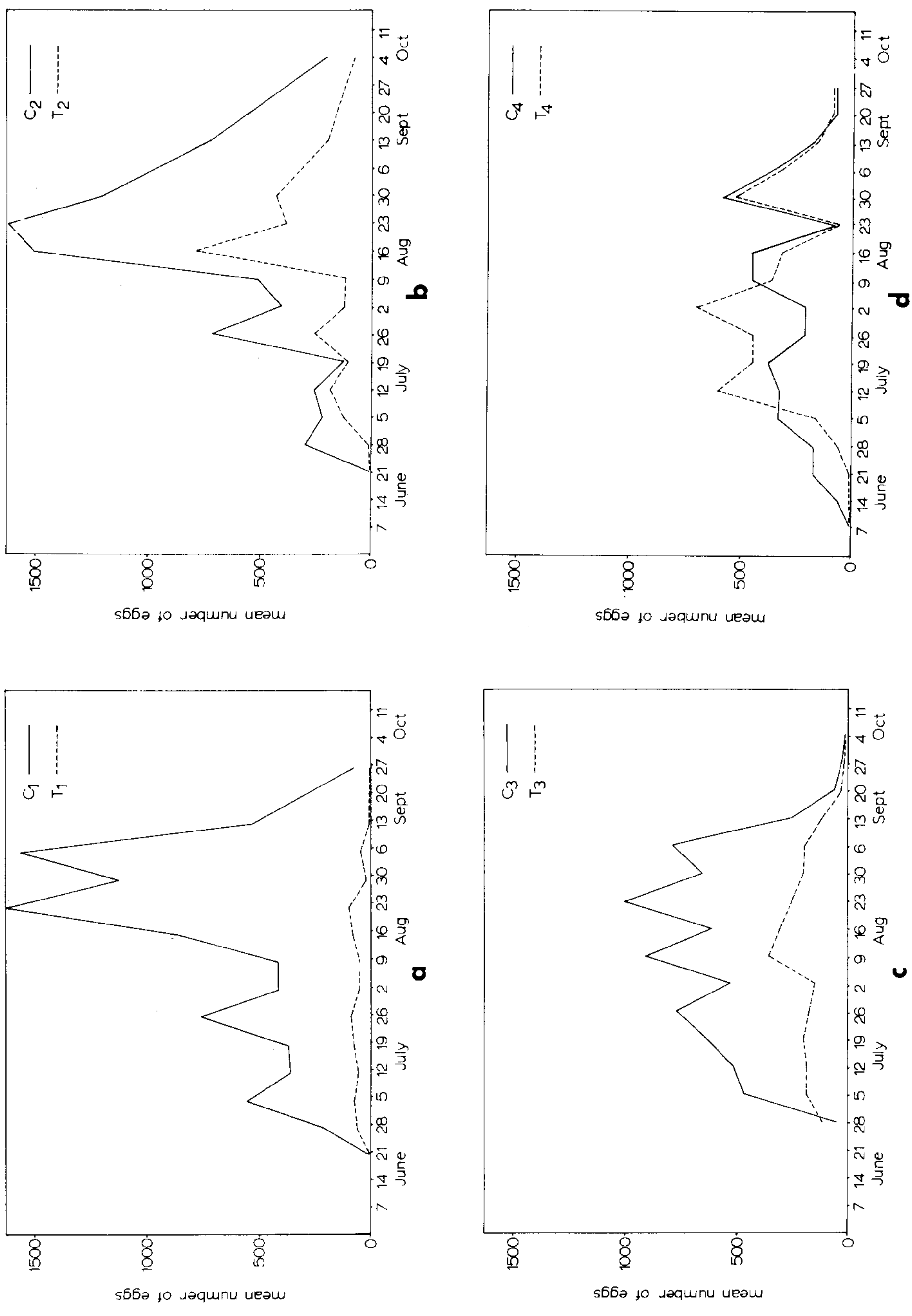

Fig. 2. Oviposition activity by *Aedes triseriatus* at 4 paired study sites in southwestern Wisconsin.

the same week as in T1 and decreased in late summer at a much more rapid rate than in C1. The 2 major peaks in Area S occurred in the 2nd and 4th weeks of July, while in the north-facing woods the 2 major peaks occurred in the 3rd week of August and the 1st week of September.

1972: Areas C2 and T2. Oviposition peaks occurred concurrently in C2 and T2 with the most prominent in the 2nd and 3d weeks of August (Fig. 2b), but the 2 areas were statistically different for the season (Table 1). Weekly comparisons were also statistically different except for the 1st 3 weeks of July which were not significantly different at the 5% level. Overall oviposition reduction was 70% if seasonal means are compared, 60% if seasonal sums are compared. Traps in T2 collected an average of 2723 eggs during the season.

Discarded tires were present in T2 in 1972 but their possible significance as a source of *A. triseriatus* was overlooked. Later analysis of oviposition in the traps closest to the tires revealed that they collected an average of 3895 eggs per trap during the season as compared to 2548 per trap over the rest of the area. This apparent increase over traps in the remainder of the area, though possibly biologically important, was not supported by statistical significance.

Traps placed in the cleared areas forming the east and west boundaries of T2 revealed that females were present there at various times during the season.

1973: C3 and T3. In C3 large successive peaks of oviposition occurred throughout the season (Fig. 2c). The T3 profile followed that of the control area less than was the case in previous years with only 1 mid-season peak coinciding with a peak in C3. The 2 areas were statistically different for the season and, specifically, for each week except the last week of June, last 2 weeks of September and 1st week of October. Overall oviposition reduction in T3 was 65.9% by both mean and sum comparisons. The average trap collection in T3 was 2502 eggs during the season.

Two areas with discarded tires and other refuse were located in T3. Each was compared separately to the rest of the area using data from traps most proximate to the rubbish site. Near rubbish site A traps averaged 3355 eggs over the season compared to 2289 eggs for non-rubbish sites. Traps near rubbish site B collected an average of 2785 eggs compared to 2431 for non-rubbish sites. Neither difference was statistically significant.

Noticeably fewer adults were attracted to the observer in T3 than in C3 early in the season so counts were made of those attracted during servicing of the ovitraps during the latter part of the season. The total number of adults counted in T3 was 8 compared to 44 in C3, a difference that appeared comparable to the 66% reduction in oviposition.

1973: C4 and T4. Eggs were first detected in both T4 and C4 traps during the 2nd week of June (Fig. 2d). Oviposition had decreased sharply in both areas by the last week of September. T4 and C4 were not statistically different over the total season though an apparent 15% oviposition reduction can be shown if seasonal sums are compared (Table 1). An apparent increase of 6.2% in T4 is shown if seasonal means are compared. Oviposition in C4 was significantly greater than in T4 at the 5% level during one week, the 3d week of June, and T4 was significantly greater than C4 at the 1% level for the last week of July.

Two rubbish sites were compared within area T4. Traps near rubbish area A averaged 4138 eggs compared to 4514 in traps not near rubbish. Traps near rubbish site B averaged 5674 eggs during the season compared to 3593 in traps not near rubbish. Neither difference is statistically significant.

Few adults were observed in either T4 or C4. When counts were made of adults attracted during the latter part of the season to the observer while servicing traps only 4 were seen in T4 and 5 in C4.

In Table 2 are presented the data on egg collections at the Kaeser sites. The trap on the isolated tree (177) collected more eggs than were collected in any other trap. The collection of 2313 eggs during the week of August 23 was the greatest number of eggs collected in a single ovitrap over a weekly period during the 3 years of the studies. To reach this trap from either wooded area required movement across grassy areas of 40 or more yards.

positing mosquitoes in areas T1 and T2 as each had open boundary lanes of less than 50 yd width on 2 sides and T1 was, in addition, connected to adjacent woods at one point by a narrow forested corridor. The sources of ovipositing females in areas T3 and T4 are not so readily obvious as these areas were 440 yd and 360 yd, respectively, from the nearest woodlands. Arboreal treeholes, i.e., above head height, are a

Table 2. Number of eggs of *Aedes triseriatus* collected per ovitrap per week in Kaeser area, 1973.

Area and trap no.	Collection for week ending								
	August				September				
	9	16	23	30	6	13	20	27	Total
South Woods									
172	1261	1070	0	0	702	144	55	69	3301
173	100	289	128	172	96	0	0	109	894
174	273	1259	1153	30	271	122	38	9	3155
175	332	401	734	714	135	72	0	0	2388
176	104	383	535	539	55	24	1	67	1708
Mean	414	680	637	381	251	72	18	50	....
Clearing									
177	356	234	2313	744	313	259	54	156	4429
North woods									
178	567	600	1287	199	145	77	20	19	2914
179	743	135	145	639	159	152	66	69	2108
Mean	655	368	716	419	152	115	43	44	....

DISCUSSION

At the beginning of this study it was expected that closure of basal treeholes in woodlots with some degree of isolation from adjacent woods would result in a greater suppression of *A. triseriatus* populations than proved to be the case. Limited early tests (Loor 1969) had failed to disclose either biting or oviposition by *A. triseriatus* in open areas even a few yards beyond the forest edge. The great number of eggs collected at the isolated tree at the Kaeser site and the finding of eggs in traps in the east and west boundary lanes of area T2 indicate, however, that this species ventures readily into the open for distances of 50 or 100 yd or more. This alone can explain the source of ovipossibility, but they do not seem to be very numerous in our study areas. A search for such cavities in 2 of our study areas (not T3 or T4) in the spring of 1974 detected many small cavities (6 cu in or less) and a few larger ones, but most were dry and only 2 contained mosquito larvae. The highest cavity with larvae was 25 ft above ground, which was near the maximum height that could be sampled with our technique, which consisted of probing cavities with a shepherd hook-like device and, when water was detected, climbing up the tree trunk.

The possible significance of arboreal treeholes cannot be dismissed, however, as they have also been observed in eastern Wisconsin (P. R. Grimstad, University of Wisconsin, unpublished data) and in Ohio

(M. A. Parsons, Ohio Department of Health, personal communication) and *A. triseriatus* has been captured in light traps in northern Wisconsin at localities where treeholes could not be found (G. B. Craig, Jr., University of Notre Dame, personal communication). Also, Loor and DeFoliart (1970) have shown that *A. triseriatus* will oviposit in traps 20 ft above ground and Garry and DeFoliart (unpublished data) have found oviposition in traps 30 ft above ground.

Rubbish heaps, often including discarded tires, were found to be of common occurrence in farm woodlots. In 4 of 5 instances, traps near rubbish sites averaged substantially more eggs than did other traps although the differences were not statistically significant. LaCrosse virus has been demonstrated to overwinter in *A. triseriatus* eggs deposited in old tires (Watts et al. 1974), and removal of tires should be included in any plan to reduce breeding by *A. triseriatus*. Cans and broken bottles inspected in rubbish sites were never found with larvae and were in most instances thought to contain water which would not attract oviposition or support larval development because of high concentrations of rust or other sediment.

Rainfall appeared to influence control. In the year when control was most successful, 1971, summer rainfall was less than in either of the other 2 years. In 1973 when the least successful control was obtained rainfall was 62.5% greater than in the corresponding period of 1971.

The data obtained indicate that closure of basal treeholes will usually result in a significant reduction of the *A. triseriatus* population in a given woodlot, but whether this reduction will ordinarily be sufficient to interrupt transmission of LaCrosse virus remains unknown Basal treehole closure may, however, introduce an additional factor that tends to reduce circulation of the virus in nature. Virus overwinters in eggs in basal cavities (Watts et al. 1974), and preliminary data for the winter of 1973–1974 (C. E. Garry, unpublished data) indicate that 10% to 15% of the basal cavities contained eggs with virus in 5 areas studied. Since arboreal treeholes appear to be relatively scarce, basal treehole closure in a given area should, therefore, significantly reduce the amount of virus available via overwintering mosquito eggs.

Until more is known about the total effects of basal treehole closure in reducing LaCrosse virus circulation in nature, research on other methods is needed. In locations where basal treehole closure is considered warranted, breeding in arboreal treeholes that cannot be easily detected from the ground, breeding in refuse such as old tires unless these are found and removed, and infiltration from surrounding wooded areas unless these are separated from the closed area by several hundred yards of open terrain appear to be the main factors responsible for reducing the effectiveness of the method.

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