Evaporation and precipitation over land and sea, connected by streams and atmospheric exchange, constitute the vast hydrologic cycle. To alter the course of this great process, man must apply his small efforts at a strategic juncture in some major component of the cycle. For example, the rainmaker hopes to change the income from rain by adding a few but sufficient crystals to clouds. Outgo, however, is our concern in this paper. We hope to show a modification of the hydrologic cycle caused by a decrease in the evaporation from plants. Certainly this evaporation is a major component of the cycle, for two thirds of the rainfall on this nation returns to the atmosphere by evaporation, mostly from leaves. The strategic juncture is provided by the microscopic pores, or stomata, in the leaves through which the transpired water diffuses. Here we report that spraying about 3 kilograms of substance per hectare upon 16-meter pine trees shrank their stomata enough to decrease significantly the evaporation portion of the hydrologic cycle locally.

Before reporting the experiment, the nature of stomata, their role in evaporation, and means for shrinking them may warrant review. In red pine (*Pinus resinosa* Ait.) needles, stomata provide 5000 holes/cm² (Fig. 1). Closure occurs at the bottom of a pit about 20 μ deep when walls of the guard cells press together. When these walls draw apart, a pore as wide as 11 μ may open (Fig. 2). Countless variations on the number and dimensions of the pores are, of course, found. Through these pores passes the carbon dioxide required for photosynthesis. When the pores have been shrunk, however, the loss of water has decreased relatively more than the gain of carbon dioxide.¹

Different opinions still are held concerning the effect of stomatal size upon evaporation from even a single leaf. Fortunately, the discovery of chemicals² that can narrow the stomata without damaging the leaf now permits controlled experiments. These have clearly demonstrated that evaporation from a single well-ventilated leaf is decreased by stomatal shrinkage throughout the range of stomatal widths.³

Numerous substances will close stomata. Several do so by altering the ability of the stomatal guard cells to accumulate water and to open the pores.³ One of these, phenylmercuric acetate, is outstanding because it lasts and because it acts at low concentrations.³ Also, phenylmercuric acetate has been shown to decrease transpiration of small isolated conifers.⁴ ⁵ It was therefore chosen for the experiment. The goal of the experiment was, of course, to test whether stomatal shrinkage or even natural differences among stomata affect the hydrologic cycle, not to test a particular substance that is a model of other substances.

Thus, at the outset of the experiment, we had a means of shrinking stomata that had decreased the evaporation from a single well-ventilated leaf in a chamber and from small isolated trees. Things are different, however, in a forest. There evaporation is often limited by the supply of energy or by ventilation,⁶ and a multitude of leaves cover each land area. The following paragraphs report whether the
shrinkage of microscopic stomata that decreased transpiration in physiological experiments would change the large and complex hydrologic cycle of a real forest.

*Experimental Procedures.—The plantation:* The plantation is in Voluntown, Connecticut. (The Connecticut Forestry Division generously permitted us to use the plantation.) It extends more than 30 m on each side of the experimental region and is surrounded by hardwood forest. The trees were 25 years old and were 16 m tall with a mean trunk diameter of 18 cm at a height of 150 cm. Planting distances were $2 \times 2$ m, and the canopy was closed. The soil is loamy sand. When we dug to a depth of 230 cm, roots were found at all depths.

*Soil water measurement:* The difference in evaporation from a portion of the forest with narrow stomata and from the untreated forest was detected by measuring the change in soil moisture. This method had the advantages of permitting replication within 2 ha and leaving the roots in their natural state. A micrometeorological method would have required larger plots and less replication, and lysimeters would have required modifying the root distribution. The change in soil water is the algebraic sum of rain, evaporation from foliage and soil surface, and migration within the soil body. Rainfall is the same upon all plots, and migration will not be less in treated than in untreated plots. The difference between the time change of soil water beneath the treated and untreated trees is, therefore, a conservative estimate of the effect of stomatal shrinking upon evaporation.

The neutron-scattering method of soil water measurement, which was employed here, permits repeated measurement at the same place. Although soil water varies erratically from place to place, the variability of change at one place is much less. Hence, the neutron-scattering method permits usefully precise measurements of soil moisture change.7 The calibration of the instrument was verified in the plantation by simultaneously counting and measuring bulk density and gravimetric water content of the soil. A change of 450 counts per minute corresponded to a change of 1 volumetric percentage of water in the soil.

Sixteen rows of access tubes for the neutron probe were arranged within the plantation. In each row were four tubes that reached a depth of 183 cm and two that reached a depth of 318 cm. Each tube was 60 cm from a dominant or codominant tree surrounded by sound trees. At the
time of treatment (June 2, 1966), and on June 16, July 8, August 11, September 2, and October 4, soil moisture was measured at 30-cm intervals within the tubes. The water near the soil surface was also estimated with a surface gauge, but these changes were less interesting because of rainfall and evaporation from the surface.

_Treatment:_ The 16 rows of tubes were divided into eight adjacent pairs, and one of each pair was selected for treatment by flipping a coin. On June 2, 3700 liters of water containing 300 ppm phenylmercuric acetate and 0.1% Triton B1956 (Rohm and Haas, Philadelphia) were sprayed upon 570 trees on 0.3 ha. The spray was applied from the ground by a hydraulic sprayer jet. Shoots were pruned from the crowns, and wetting of foliage was thus verified. Analysis of the foliage by L. G. Keirstead of the Station's Analytical Chemistry Department revealed 4 to 19 ppm Hg in the treated and 0.1 or less ppm in the adjacent untreated foliage. At the time of treatment, the new internodes were about 7 cm long, but the new needles were mere scales.

_Response of the trees:_ Stomatal narrowing or shrinkage was detected by infiltrating an organic solvent into sunlit leaves. Differences among the trees were estimated objectively by ranking the needles from most to least infiltrated. The solvent contained a dye, and its infiltration is evident in Figure 1.

The contraction of the trunk or bole between 0600 and 1300 hours on sunny days is caused by the daily dehydration of the trees during rapid transpiration. Presumably, a decrease in transpiration of evaporation from the foliage would make the daily dehydration less and this in turn would make the contraction smaller. The contraction was measured with a dendrometer: three nails were driven into the heartwood, and a metal disk was cemented to sound bark in the center of the triangle formed by the nails. A mechanical depth gauge measured the distance between the heads of the nails and the metal dish. An hour was required to measure all, less than 10 minutes to measure adjacent rows of six treated and six untreated trees. The specific dendrometer employed was developed and tested in earlier investigations by Bravdo and R. M. Samish.

Growth of the tree was indicated by the expansion of the bole radius between morning and afternoon evaporation. Presumably, a decrease in infiltration was, of course, less in treated than untreated, offering evidence of stomatal shrinking. Although the stomata shrank, they were not closed. That is, even treated needles admitted more solvent than needles whose stomata had been closed by darkness.

On July 7, needles were collected from 24 branches in two pairs of plots. In both pairs of plots, the treated admitted less solvent than the untreated. On August 26 and September 9, no evidence of stomatal shrinking could be found in 1965 or 1966 needles.

_Diurnal contraction of bole:_ During sunny days in June, July, and early August, boles contracted significantly less where the stomata were treated than where they were not.

<table>
<thead>
<tr>
<th>Date</th>
<th>Evap.</th>
<th>Check</th>
<th>Dif$</th>
<th>Diff ± 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 17</td>
<td>0.08</td>
<td>86 ± 5</td>
<td>18 ± 8</td>
<td>0.07</td>
</tr>
<tr>
<td>June 23</td>
<td>0.22</td>
<td>86 ± 3</td>
<td>22 ± 4</td>
<td>0.20</td>
</tr>
<tr>
<td>July 7*</td>
<td>0.17</td>
<td>78 ± 4</td>
<td>22 ± 4</td>
<td>0.20</td>
</tr>
<tr>
<td>July 12</td>
<td>0.34</td>
<td>96 ± 4</td>
<td>32 ± 13</td>
<td>0.20</td>
</tr>
<tr>
<td>Aug. 12</td>
<td>0.16</td>
<td>101 ± 4</td>
<td>22 ± 6</td>
<td>0.20</td>
</tr>
<tr>
<td>Aug. 25†</td>
<td>0.20</td>
<td>84 ± 7</td>
<td>2 ± 6</td>
<td>0.20</td>
</tr>
<tr>
<td>Oct. 4</td>
<td>0.07</td>
<td>37 ± 2</td>
<td>4 ± 3</td>
<td>0.20</td>
</tr>
</tbody>
</table>

* Mean change on July 7 and 8.
† Mean change between morning Aug. 26 and afternoon Aug. 25 and 26.
‡ Standard error of mean of 40 observations.
§ Difference and standard error of difference, means for 40 check minus 40 treated trees.
were untreated (Table 1). The decline of the difference in late August accompanied the expansion of new needles and our inability to detect stomatal shrinkage in either new or old needles in late August.

**Growth of trees:** Frequent examination of needles revealed no injury to foliage by phenylmercuric acetate. Neither the length nor weight of the 1966 needles relative to the length or weight of the 1965 needles on the same branch was affected by treatment.

The expansion of the bole was, on the other hand, affected by treatment. Increments shown in Table 2 are independent of bole diameter. They show the great expansion during July and early August. During three of the five intervals, the trees with shrunken stomata expanded less than those with wide stomata. The total expansion for the 109-day season was decreased 15 per cent by treatment.

**Soil water:** The preceding sections have described the shrinking of stomata in the 1965 needles during June and July, the lessened diurnal contraction of the stems, and the decreased seasonal stem enlargement that followed a spray which did not visibly injure nor decrease the expansion and weight of 1966 needles. These are the “side effects.” We now turn to the primary subject: any decrease in evaporation revealed by the expenditure of soil water.

Let us first examine the general behavior of soil water. The depletion of water in the upper soil was estimated from observations at 30, 61, . . . , 183 cm (Fig. 3). Each point for each curve represents the mean sum of 48 sums of six observations in a tube. The near equality of the water near the paired tubes at the time of treatment, the rapid depletion of water from mid-July to mid-August, and the wetting of this portion of the profile during September are all evident.

The depletion of water from the lower soil was estimated from observations at 213, 244, . . . , 335 cm (Fig. 4). Here, each point represents the mean sum of 16 sums of five observations in a tube. The initial gain of water (presumably leaching from above) and then the depletion even during September rains are evident. The content of water in the lower soil did not affect the rate of depletion in the upper soil.

If to these changes in stored water are added the small changes indicated near the surface, plus the rainfall measured by a gauge at crown height, estimates of total evaporation from the plantation are obtained. The mean rates during the five periods range from 6 mm/day in the middle to 2 in the last period. Relative to open water evaporation, the loss from the forest was rapid during the first and third intervals. (Observations of evaporation from Class A pans at nearby Storrs, Connecticut, and Kingston, Rhode Island, were furnished by B. E. Janes and R. C. Wakefield.) Likely, the first event was caused by a significant amount of leaching beyond 335 cm and the later event by the opening of stomata in the 1966 needles.
Having dealt with the general nature of the change in soil water, we can now examine the effect of the stomatal shrinkage. The courses of water depletion in the surface and lower soil revealed no effects and can be quickly dismissed. That is, analyses of variance revealed no evidence of effect of treatment upon changes in the soil water near the surface or below 200 cm. The greater mean water content of the lower soil beneath treated trees than beneath untreated trees (Fig. 4) was due to chance choice of plots and was statistically insignificant. The mean square of the interaction of treatment and blocks was 177 times that of the interaction of time, treatment, and blocks. Hence, the mean contents may differ considerably without significance, while small differences in the slopes of the lines in the figures may be significant. These slopes, however, were not different in the 213- to 335-cm zone.

In the 30- to 183-cm zone where roots were numerous and where depletion of soil water was greater and earlier, treatment did save water and change the slopes. In an analysis of variance of all 3456 observations of water at the six dates and six depths in this zone, the mean square for the effect of treatment upon the change of water was fully 24 times the mean square for its variation either among or within the eight pairs of rows. Because the analysis of variance is robust, it is sound to infer that stomatal shrinking decreased evaporation and saved a significant amount of water in the 30- to 183-cm soil region. Before considering the saved quantity, however, the heterogeneity of variance must be examined.

At each depth in each time interval, observations were made in 48 pairs, an individual for a treated and an individual for an untreated site. The difference be-
TABLE 3

SAVINGS OF SOIL WATER AND STANDARD ERRORS IN MILLIMETERS

<table>
<thead>
<tr>
<th>Depth (ft)</th>
<th>June 3–16</th>
<th>June 17–July 8</th>
<th>July 9–Aug. 11</th>
<th>Aug. 12–Sept. 2</th>
<th>Sept. 3–Oct. 4</th>
<th>June 3–Oct. 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.1 ± 0.8</td>
<td>3.0 ± 2.3</td>
<td>-6.5 ± 2.6</td>
<td>0.2 ± 0.6</td>
<td>3.1 ± 1.6</td>
<td>2.8 ± 1.3</td>
</tr>
<tr>
<td>2</td>
<td>3.2 ± 0.8</td>
<td>4.1 ± 1.6</td>
<td>-4.7 ± 2.1</td>
<td>0.9 ± 0.7</td>
<td>-3.9 ± 2.0</td>
<td>-0.3 ± 1.6</td>
</tr>
<tr>
<td>3</td>
<td>1.2 ± 0.9</td>
<td>0.6 ± 1.0</td>
<td>2.0 ± 1.6</td>
<td>2.1 ± 1.3</td>
<td>-1.0 ± 1.7</td>
<td>4.7 ± 2.4</td>
</tr>
<tr>
<td>4</td>
<td>0.6 ± 0.9</td>
<td>0.4 ± 0.9</td>
<td>1.6 ± 1.4</td>
<td>1.2 ± 1.4</td>
<td>-0.4 ± 2.4</td>
<td>3.4 ± 3.5</td>
</tr>
<tr>
<td>5</td>
<td>1.8 ± 0.9</td>
<td>1.4 ± 0.7</td>
<td>0.5 ± 1.4</td>
<td>-1.6 ± 1.2</td>
<td>5.9 ± 1.8</td>
<td>8.1 ± 2.9</td>
</tr>
<tr>
<td>6</td>
<td>-0.4 ± 0.8</td>
<td>0.4 ± 0.6</td>
<td>0.5 ± 1.5</td>
<td>0.1 ± 1.4</td>
<td>1.0 ± 1.1</td>
<td>1.6 ± 2.5</td>
</tr>
<tr>
<td>Sum</td>
<td>9.5 ± 1.7</td>
<td>9.9 ± 3.8</td>
<td>-6.6 ± 5.0</td>
<td>2.9 ± 2.9</td>
<td>4.6 ± 4.1</td>
<td>20.3 ± 6.6</td>
</tr>
<tr>
<td>Weighted sum</td>
<td>9.3 ± 2.1</td>
<td>5.8 ± 2.2</td>
<td>0.2 ± 4.0</td>
<td>2.7 ± 2.2</td>
<td>6.7 ± 4.0</td>
<td>28.2 ± 5.7</td>
</tr>
</tbody>
</table>

between the treated and untreated individuals of the pairs at the beginning less the difference at the end of a time interval are, of course, estimates of the effect of stomatal shrinking upon evaporation. The mean and standard errors of these differences attributed to treatment were calculated for the 30 depth and time combinations and for the seasonal differences at the six depths (Table 3). Because the variances are heterogeneous, the estimation of total saving is difficult.

First, each observation of a saving throughout the 30- to 183-cm profile between June 3 and October 4 may be given equal weight. A significant saving of 20 mm with standard error of 7 is then estimated.

The more variable increments may be given less weight and the less variable ones more weight. If each of the 30 estimates of savings at a depth and in an interval is weighted by division by its variance, the weighted sums at the bottom of Table 3 are obtained. These show significant savings in the first two intervals, June and early July. Although savings predominate during the remainder of the season, they are insignificant. Finally, the weighted sum of the savings throughout the season is 28 mm with a standard error of 6. Thus whether the savings are weighted equally or according to their variance, a substantial and significant decrease is estimated in the evaporation portion of the hydrologic cycle.

Discussion.—Two questions to be answered during the continuing pine experiment are clear. The first concerns the long-term effect upon the pines. One would wish that these effects would be of stomatal narrowing alone. Unfortunately, some of the effects will be of phenylmercuric acetate apart from its stomatal controlling characteristics. Phenylmercuric acetate has been employed for a quarter century as an eradicant of the scab fungus in apple leaves. Its yellowing of leaves, especially in hot weather, is general knowledge. The sum of this toxicity plus any limiting of carbon dioxide diffusion by stomatal shrinking should not be ascribed to stomatal change alone. Nevertheless, some compound must be employed, and at least, the long-term effects of stomatal control will not be underestimated by using phenylmercuric acetate as a model.

The second question concerns increasing the change in the hydrologic cycle. Since evaporation was changed only about 5 per cent in 1966, much room remains for greater change. Greater change could be expected from greater shrinkage for the stomata at Voluntown were never closed as by darkness. Also, greater change in evaporation for the entire season should be obtained from additional treatment. After all, the single spray at Voluntown affected the old needles only in the beginning and never affected the new needles detectably. Treatment of old needles on
June 1 and of all needles in mid-July may, therefore, change the hydrologic cycle more than the significant amount that we have so far observed.

Confirming another role for plants in the hydrologic cycle is the chief aspect of these results deserving discussion. The necessity of a canopy of well-watered, green plants rather than sun-parched soil has long been recognized in definitions of potential evaporation. Given some kind of healthy canopy, however, the major role in evaporation control was given to radiation with a much smaller role for vegetation.6 Recently, evidence of differences in evaporation among different sorts of vegetation has become clear, and these differences have been attributed to different colors and absorption of radiation, to different aerodynamic roughnesses, or to different interceptions and rapid evaporations of rain.9 In the Voluntown pines, on the other hand, differences in reflection, in roughness, or in interception of rain between treated and untreated trees are difficult to conceive, and the modest narrowing of stomata caused by a single spray must have caused the significant change in evaporation.

Testing whether cloud seeding increases rain has been plagued by extremely difficult statistical problems. Fortunately, testing whether stomatal shrinkage changes the hydrologic cycle has been easier. Replicated plots in a small area, random assignment of treatment to one of a nearly identical pair, and repeated measurement of water at the same place are advantages of stomata shrinking that the rainmaker will envy. Aided by these advantages, we have observed a significant change in evaporation that has produced the same increase in soil moisture in October as if 20 to 28 mm of rain had fallen on the treated plots.

Summary.—Shrinking stomata in the needles of 16-m pine trees by a single spray of 300 ppm phenylmercuric acetate on June 2 decreased the contraction of the tree boles that is caused by dehydration during rapid transpiration. It did not stunt the needles but did decrease growth of the bole 15 per cent. Finally, the decrease in evaporation caused by stomatal shrinking was sufficient to cause a 20- to 28-mm smaller depletion of soil moisture between June and October. Since this significant change was but 5 per cent change in evaporation and since stomata were affected only part of the season, these microscopic pores may provide a strategic means for changes of great scope in the hydrologic cycle.

1 Zelitch, I., and P. E. Waggoner, these PROCEEDINGS, 48, 1101 and 1297 (1962).
2 Zelitch, I., these PROCEEDINGS, 47, 1423 (1961).
4 Keller, Th., private communication (1965).