Test of a Transpiration Inhibitor on a Forested Watershed

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Abstract. The glyceryl half-ester of decenylsuccinic acid (GlOSA) closes tree stomata when sprayed directly upon the undersides of leaves. Regression analysis of streamflow from two small watersheds at Coweeta showed that a 12% reduction in transpiration might be detected as a significant increase in streamflow. Two sprays of 50 pounds of GlOSA in water applied to 30 acres of one watershed from a helicopter produced little deposit on the undersides of leaves and no clear evidence of stomatal closure. Observed increases in streamflow were statistically insignificant.

Nearly all the water plants absorb from the soil passes through the stomata or pores in the leaves. Stomatal resistance is a natural inhibitor of transpiration, partially dependent on stomatal opening. The artificial closure of stomata should decrease transpiration and increase water percolating through the soil to streams or groundwater. A harmless spray that closes stomata is being sought to increase the water yield of forested watersheds without destroying the timber crop or the protection afforded by vegetation.

Theoretically, the narrowing of stomatal pores will decrease transpiration considerably [*Zelitch and Waggoner,* 1962]. A dilute solution of phenylmercuric acetate sprayed on leaves of tobacco, maize, and cotton narrowed the stomatal pores and decreased the transpiration of plants in chambers [*Zelitch and Waggoner,* 1962; *Shimshi,* 1963*a*; *Slatyer and Bierhuizen,* 1964]. Also, the change in stomatal resistance caused by a foliar spray significantly decreased transpiration from sunflowers growing beneath a plastic roof [*Shimshi,* 1963*b*], and from barley growing in a 20-acre field [*Waggoner et al.,* 1964]. Hence, we hoped that streamflow from a forested watershed might be increased by a foliar spray that would close stomata but leave the trees intact.

Early in 1964 the staff of the Connecticut Station sought a compound that could be sprayed in low volume upon a variety of broadleaved trees to narrow the stomata without injury. The staff of the Coweeta Hydrologic Laboratory selected a pair of calibrated watersheds and prepared them for the experiment. In June 1964 one watershed was sprayed from the air. The foliage was examined and streamflow was measured with the following results.

PREPARATORY INVESTIGATIONS

Detecting stomatal opening. Few leaves have stomata upon their upper surfaces [*Salisbury,* 1927]. Dogwood stomata open as wide as 4 microns if the leaves are enclosed in a plastic bag for an hour; this is large enough for microscopic examination of stomatal replicas [*Zelitch,* 1961]. Although the ability of solutions to infiltrate stomata provides no direct measure of resistance to gaseous diffusion, infiltration is well correlated with stomatal size [*Dale,* 1961], and the method is suitable for small pores, such as are found in oak leaves. We chose to use the viscosity of the median solution that infiltrated stomata in 15 to 30 seconds as an index of stomatal opening. Pure acetone provided this lowest viscosity; higher viscosities were provided by ethanol with 0, 1, 2, 8, and 18% water added.

TABLE 1. Stomatal Opening in Chestnut Oak Detected by Infiltration One Day after Spraying with GlOSA in 0.2% Triton

Leaf Number	Molarity GlOSA	Side Sprayed	Median Viscosity* Infiltrating
1–3	0.01	Bottom	0.3
4,5	0.01	Bottom	0.3
6–8	0	Neither	1.3
9,10	0	Neither	2.1
11–13	0.1	Top	1.3
14,15	0.1	Top	1.3
16–18	0	Neither	2.1
19–21†	0.1	Bottom	0.3
22,23†	0.1	Bottom	0.3
24–26	0	Neither	2.1

* Expressed in centipoise at 20°C.
† Leaf severely injured; infiltration observed in a sound portion of leaf.

Chemical for stomatal closure. Phenylmercuric acetate, although highly effective, severely injured tree leaves and could not be considered. The alkenylsuccinic acids decrease transpiration by stomatal closure [*Waggoner et al.*, 1964], and decenylsuccinic acid is most effective in closing stomata [*Zelitch,* 1964]. Its glyceryl half-ester (GlOSA) was the least toxic of several esters when 0.01 *M* suspension in 0.2% Triton B1956 (a wetting agent, Rohm and Haas, Philadelphia) were sprayed on the undersides of red oak leaves, and infiltration demonstrated stomatal closure without visible injury. Seventeen hours exposure of 10^{-3} *M* GlOSA in a shallow container to ultraviolet radiation (lamp peak efficiency, 366 micron) considerably diminished its ability to close stomata.

In June, the leaves of chestnut oak sprouts at Coweeta were sprayed with GlOSA in 0.2% Triton until the solution ran. The molarity of GlOSA and the median viscosity of solutions that would infiltrate the leaves on the following day are shown in Table 1. The stomata of leaves sprayed underside showed considerable closure. One month later tests showed that the effect was still present.

Further tests at Coweeta showed narrowed stomata and decreased water loss from severed dogwood leaves previously sprayed to saturation with GlOSA. Even Triton alone closed some of the stomata, but, on leaves sprayed with 0.01 *M* GlOSA, 85% of the stomata were closed and vapor loss was 50% less than from unsprayed dogwood leaves. Clearly, the stomata of chestnut oak and dogwood (about ⅓ of the total canopy in the experiment) can be closed by enough GlOSA sprayed directly on the stomata. It should be noted, however, that saturating sprays stronger than 0.002 always caused some brown spots on dogwood leaves.

Experimental watershed. The experimental watersheds at the Coweeta Hydrologic Laboratory in western North Carolina have been described many times [*Hewlett and Hibbert,* 1961]. A calibrated weir with a concrete cutoff wall catches practically all water from the experimental area. The adjacent pair, designated watersheds 19 and 21, selected for this test was used in an understory cutting experiment in 1948 [*Johnson and Kovner,* 1956]. By 1955 increases in streamflow due to cutting were negligible. Eliminating the period 1948–1955 leaves a 15-year calibration record of rainfall and streamflow.

Both watersheds measure about 70 acres, rise about 1000 feet from the weir to the summit, and face north at about 40% slope. The cover

TABLE 2. Summary of Plant Cover within the 30-Acre Treatment Area (basal area per acre averaged 85 square feet)

Strata	Species	Cover,* %
Canopy (dominant and codominant trees)	Chestnut oak	25
	Other oaks	10
	Hickory	8
	Red maple	4
	Black gum	3
	7 other species	6
	Total	56
Subcanopy (intermediate and suppressed trees)	Red maple	13
	Black gum	11
	Dogwood	9
	Chestnut oak	8
	12 other species	13
	Total	54
Shrubs	Laurel-rhododendron	55
Ground cover	*Gaylussacia* spp.	30
	Other species	6
	Total	36
Total Vertical Cover, all strata		100

* Cover averaged from 48 plots (16% of total area) by ocular estimation of vertical crown cover.

is typical of the dense forest of the southern Appalachians. The upper crown level, from 60 to 80 feet above the ground, is ragged, owing partly to the death of chestnut in the 1930's. Table 2 shows the character of the cover, dominated by oaks, red maple, black gum, and laurel-rhododendron shrub.

Increases in water yield produced far upslope from the stream channel are released over a long time and are difficult to measure hydrologically. To increase chances of a favorable response and to reduce the cost of the experiment, we sprayed 30 acres (3/7) of watershed 19 along the slopes adjacent to the stream. Watershed 21 served to predict the flow on watershed 19 after treatment. The actual flow minus the predicted flow estimated the treatment effect, which we expected to be positive.

We hoped for measurable response in the diurnal fluctuation due presumably to transpiration along the riparian strip, the instantaneous flow at midnight, and the total yield by months. Previously, riparian forest cutting had temporarily eliminated diurnal fluctuation [*Dunford and Fletcher,* 1947]; other treatments have increased instantaneous flow and total water yield [*Hewlett and Hibbert,* 1961].

Calibration regressions of watershed 19 on watershed 21 are shown in Table 3. The symbols are:

csm, streamflow measured in cubic feet per second per square mile.

inch, streamflow by periods in inches applied to the entire watershed.

R_7, antecedent 7-day rainfall in inches.

F_t, diurnal fluctuation on watershed 19, calculated by subtracting minimum flow in csm on a *non-storm day* from the average of the two midnight flows before and after this minimum (t represents treatment).

F_c, simultaneous fluctuation on watershed 21 (c represents control).

M_t, midnight instantaneous flow in csm on watershed 19 during non-storm periods.

M_c, simultaneous midnight flow on watershed 21.

S_t, total monthly streamflow in inches on watershed 19.

S_c', S_c'', S_c''', total monthly streamflow on watershed 21 (June, July, August).

T_t, total trimonthly streamflow (June–August, inclusive) in inches on watershed 19.

T_c, total trimonthly streamflow on watershed 21.

N, number in sample.

r, correlation coefficient.

$sy \cdot x$, standard error of estimate by regression.

Summarizing Table 3, diurnal fluctuation can be predicted within 75% of its true value about 95% of the time, and instantaneous flow and total monthly yield within 15% about 95% of the time. The average trimonthly flow from June to August is 9.5 inches and calculated potential evapotranspiration is about 13.5 inches. If we neglect the lag between decreased vapor loss and increased streamflow, trimonthly regression analysis should detect a 0.7-inch increase (0.05 level of confidence) in streamflow

TABLE 3. Regression Equations, Watershed 19 on 21, Calibration Period

Period	Prediction Regression	N	r	$sy \cdot x$, csm
June	$F_t = 0.055 + 0.765F_c$	265	0.77	0.049
June	$M_t = + .238 + 771M_c + .107R_7$	265	.98	.172
July	$M_t = - .025 + .891M_c + .093R_7$	296	.95	.189
August	$M_t = + .041 + .850M_c + .089R_7$	311	.96	.147
				inch
June	$S_t = 0.506 + 0.771S_c'$	15	0.96	0.288
July	$S_t = .336 + 1.062S_c''$	15	.98	.189
August	$S_t = .037 + .941S_c'''$	15	.99	.171
June–August	$T_t = .502 + .699S_c' + .914S_c'' + 1.010S_c'''$	15	.99	.317

on the whole watershed, equivalent to a 1.6-inch decrease in vapor loss on only 3/7 (30 acres) of watershed 19.

Could we expect GlOSA to reduce vapor loss 1.6 area inches in three months? Vapor loss from dense forest is nearly all transpiration or interception loss. Vapor loss during this experiment occurred mostly as transpiration, because little rain fell. Dividing 1.6 inches by the expected normal transpiration (13.5 inches), we calculated that a 12% reduction in transpiration on 3/7 of the area should be detected as a significant increase in streamflow. Experiments by *Waggoner et al.* [1964] led us to hope for at least 10% reduction in transpiration due to stomatal closure. Therefore, chances for detection of an increase were slight, but the importance of any increase and the uncertain effect of the spray upon a woodland called for a pilot test.

SPRAYING

On June 9, 1964, indicator papers were distributed about the watershed. Strips about 5 by 50 millimeters were attached to the upper and lower surfaces of 14 leaves at heights of 2 to 3 meters. Other strips were suspended at intervals from the ground to about 16 meters at three locations. Finally, 120 papers were suspended on 24 transects that crossed the boundary of the 30 acres to be sprayed. Also, 6 microscope slides coated with carbon were exposed. The papers indicated distribution of the spray on the top and bottom of the leaves, in the vertical plane, and over the watershed; the smoke slides revealed droplet size.

Under a clear sky and with little wind, 150 gallons of 0.1 *M* GlOSA in 0.2% Triton were applied to 35 acres between 0713 and 0824 EST. The acreage sprayed was arbitrarily established as the area within which sensitive papers received 2 drops per cm^2. Application was by helicopter leased from the Tennessee Valley Authority by the Connecticut Station, and the GlOSA was furnished by the Humphrey Chemical Company, North Haven, Connecticut. The same quantity of GlOSA in 300 gallons of solution was applied similarly on June 23.

RESULTS

The craters in the smoke slides had a median diameter of about 150 microns; if the diameters are weighted by volume, half the volume produced craters larger than 300 microns. The median interception of droplets by papers suspended at a height of from 1 to about 16 meters at 1-meter intervals was 13 and 15 per cm^2 on June 9 and 23. There was no clear trend of interception with height. On the upper sides of the leaves, the median density of droplets per cm^2 was 42 and 67 on the two occasions. On the underside, however, no droplets struck 10 of 13 papers on June 9, and 11 of 16 papers on June 23.

During June, the sprayed vegetation and the water in the weir were inspected frequently. Brown spots were found on the youngest leaves of tulip poplar, and some leaflets fell from black locust trees.

Several branches of dogwood leaves were protected in plastic bags during both sprayings; the bags were removed after spraying. On three mornings in late June, these branches were again enclosed in bags to enlarge stomatal opening; adjacent continually exposed leaves were also enclosed. After 90 to 180 minutes of enclosure, stomatal replicas were made simultaneously each morning. Stomatal width was not consistently decreased by exposure to spray from the helicopter.

Protected and sprayed leaves of the dominant species (chestnut oak) were severed and weighed in the woods on a torsion balance. The loss in weight while the leaves were suspended on the balance for 3 minutes was assumed to be an index of normal transpiration. Small differences between sprayed and protected leaves provided no conclusive evidence of a reduction in transpiration.

Finally, the relative water deficits [*Hewlett and Kramer*, 1963] of chestnut oak and dogwood leaves on the sprayed and adjacent unsprayed watershed were determined. Differences were generally less than the standard error. At midday two and nine days after spraying, the means for dogwood on the unsprayed watershed were 8.8 and 9.7%.

The deposit of 0.1 *M* GlOSA from the helicopter onto the underside of leaves was negligible. No convincing evidence was found of narrowed dogwood stomata, of decreased diffusion of water from chestnut oak leaves, or of increased hydration in leaves, despite the known ability of GlOSA to close chestnut oak and dogwood stomata.

TABLE 4. Differences (Actual Minus Predicted) in Diurnal Fluctuations and Midnight Flows in the Sprayed Watershed, by csm Classes
(See Table 3 for comparison with $sy \cdot x$.)

Period	csm Classes						
	−0.06 −0.10	−0.01 −0.05	0	0.01 0.05	0.06 0.10	0.11 0.15	0.16 0.20
	Number of Days						
June fluctuation	1	4	1	6	4	0	0
June midnight flow	0	3	1	7	5	0	0
July midnight flow	0	5	1	5	4	2	0
August midnight flow	1	3	0	2	4	5	3

Applying the calibration regressions in Table 3 to streamflow during June to August 1964, we found no firm basis for suspecting that flow increased or that diurnal fluctuations were reduced (Tables 4 and 5). No differences between predicted flow and actual flow were significant. Midnight streamflow differences after spraying were more frequently positive than negative, possibly because samples of days that follow each other are not strictly independent samples, as required by assumptions underlying regression; i.e., the successive events tend to be correlated. In any case, estimates of flow are only as good as indicated by the standard error of regression, and on this basis there was no evidence of treatment effect.

CONCLUSION

Despite the ability of an alkenylsuccinic acid to close stomata and decrease transpiration in barley, the application of more than twice as much of the compound per acre to woodland failed to increase streamflow significantly. Perhaps stomatal resistance is unimportant in woodland evapotranspiration, perhaps streamflow was increased but too little to be detected by hydrologic analysis, or perhaps ultraviolet radiation destroyed the chemical. More likely, we were unable to close a significant number of the many types of stomata by spraying from above.

Although the helicopter flew near the tops of the tallest trees and fluttered the uppermost leaves, few drops struck the underside of the leaves of the many trees that were shorter but nevertheless sunlit. Since the material is not translocated well from upper to lower leaf surfaces and since most trees have no stomata in upper leaf surfaces, failure to close stomata is not surprising. Therefore, an adequate test of the effect of stomatal closure upon streamflow must await a means of delivering spray to the underside of upper leaves, a translocatable material that closes stomata, or perhaps more sensitive tests for detecting small changes in streamflow.

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TABLE 5. Increases (Actual Minus Predicted) in Total Yield from Sprayed Watershed, by Monthly and Trimonthly Periods (in inches)

	June (6–9 through 7–8)	July	August	June–August
Increase	+0.10	+0.14	+0.16	+0.12
$sy \cdot x$	0.29	0.19	0.17	0.32

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