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Effects of Changing Stomatal Width in A Red Pine Forest on Soil Water Content, Leaf Water Potential, Bole Diameter, and Growth

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Abstract. Spraying a 16 meter tall stand of red pine (*Pinus resinosa* Ait.) with 10^{-3} M phenylmercuric acetate in early June and again in mid-July resulted in the water use between June 1 and October 25 being reduced by almost 10 %. It was demonstrated that this was caused by an increase in the leaf resistance with partial stomatal closure, which reduced absolute water potential in the needles by 1 to 3 bars in the middle of the day. Smaller demands were made upon the reserves of water in the bole of the tree as shown by the smaller bole contraction in the treated trees. Although needle length and dry weight were unaffected by the spray, radial growth was reduced by approximately 32 %. The dependence of leaf resistance on light intensity is shown, and its independence from leaf water potential discussed.

Chemical means of increasing the stomatal resistance have been known for some years (17) and have been used to study the effects of stomatal aperture on transpiration. Since the stomata regulate not only the rate of water loss but also the exchange of carbon dioxide, the effects on photosynthesis and growth have also been followed. Zelitch and Waggoner (18), Shimshi (9), and Slatyer and Bierhuizen (12) have shown that small concentrations of phenylmercuric acetate (PMA) decreased both transpiration and growth but the effect was greater on transpiration, thus improving the efficiency of water use by the plants. However, Keller (6) found that spraying small spruce trees with 10^{-3} M PMA reduced CO_2 uptake significantly more than it decreased transpiration, and also stimulated respiration.

In pine, studies have been conducted since 1962 at The Connecticut Agricultural Experiment Station to determine the effect of PMA on transpiration. The pines were grown in pots, cans, and lysimeters, and in each case, reductions in transpiration were recorded (14). During 1966, the role of the stomata in evapotranspiration and growth was taken a stage further by Waggoner and Bravdo (15) who sprayed a stand of red pine with PMA. They demonstrated that a single spray in June resulted in a 20 to 28 mm smaller depletion of soil water between June and October. They also observed a 15 % reduction in the radial growth of the bole over the same period. The PMA was only effective in conserving soil water for approximately 1 month. The experiment was continued to determine the longer term effects of PMA on growth and evapotranspiration. Since a new crop of needles emerges in July in red pine, a further spray of 10^{-3} M PMA was applied after the new needles appeared. A more comprehensive study of the effect of PMA on growth was undertaken and the diurnal and seasonal changes in leaf resistance, leaf water potential and bole contraction were followed.

Materials and Methods

The plantation of red pine (*Pinus resinosa*, Ait.) in which the experiment was conducted has been adequately described (15). The trees which had been sprayed in 1966 were sprayed again on June 1, June 8, and July 19, 1967 with a 10^{-3} M solution of phenylmercuric acetate (PMA), using a 0.1 % solution of Triton B-1956 (Rohm and Haas, Philadelphia, Pennsylvania) as a wetting agent. When the trees were sprayed on June 1, there was no detectable change in stomatal opening of the needles and subsequently a deposit was found in the bottles of PMA used. Standard laboratory tests with tobacco indicated that the chemical had deteriorated during 12 months in storage. A fresh batch of PMA solution was used on the later occasions.

The volumetric water content of the soil was determined using the neutron scattering method. The layout of the access tubes in the forest has been described (15). Readings were taken at weekly intervals from June 1 until September 13 and then again on October 25. On each occasion, the soil water profile at 30 cm intervals was observed in all 8 replicates except on June 22 and August 30 when heavy rain prevented complete data from being collected.

At regular intervals throughout the season, foliage from the third to fifth whorls from the tree-top was sampled and a portion of a branch placed in a pressure chamber (8). The pressure at which the xylem sap returns to the cut end of the branch was equivalent to the water potential of the leaf or needles in sunflower and yew (2). H. C.

de Roo of The Connecticut Agricultural Experiment Station confirmed that this was also true for red pine. The results presented are absolute values of leaf water potential. Thus, values increase when the leaf water potential (negative) decreases.

Simultaneously, 5 fascicles (10 needles) were removed from the same branch and inserted 4 cm into the chamber of an agitated diffusion porometer (10). This is a modification of the rate hygrometer described by Wallihan (16). The rate of increase in humidity and the air temperature in the sealed chamber were recorded. The time required for the humidity to increase by a fixed amount was observed and corrected to 20°. These times will be employed as indices of resistance of the epidermis. A minimum of 2 samples per branch was always used. Readings of resistance and potential were obtained within 5 minutes of sampling. Immediately prior to sampling, the radiation above the branch to be sampled was measured with an omnidirectional meter constructed from 2 silicon photovoltaic cells placed back-to-back inside a table-tennis ball that acted as a diffuser. This was placed near the cutter of a pole-pruner used to sample the foliage. The meter was calibrated with an Eppley pyrheliometer.

As in the previous year, the diurnal shrinkage of the bole of the trees was measured between 0800 and 1400 hours with a dendrometer. These observations were taken on the same occasions as the soil moisture determinations. Readings at 1400 hours were also obtained on April 10 and December 7.

Growth of the tree was indicated by the expansion of the bole radius between observations at 0800 hours. On October 24, a 4 mm by 25 to 30 mm core was taken from the bole of each of the 96 trees. Core samples were taken at the same height but on the opposite side of the tree from the metal dendrometer disc. The cores were immediately placed in plastic straws, sealed, and stored moist until the growth increments for 1965, 1966, and 1967 were measured.

Needle growth was measured on September 13 when both 1966 and 1967 needle samples were collected from the fourth to sixth whorl from the tree-top in 48 trees. The mean length and dry weight of 25 needles from each tree were determined.

From September 15, 1967, needles falling from the trees were collected in 96 tubs, 18.5 cm in diameter, which were placed 60 cm from the base and to the south of each tree marked for dendrometer measurements. The needles were removed on October 25 and December 7, and the dry weight and number of fascicles were determined.

Results

Soil Water. During the first week after spraying the trees on June 8, there was a large (9 mm) saving in the water lost from the upper 183 cm of the soil by evapotranspiration in the treated plots. Over the next 2 weeks, a further 6 mm of water was conserved in the soil in the treated plots. After June 22, 3 weeks from the application of PMA, there were no further savings until the trees were sprayed again on July 19 (fig 1). This additional spray caused a saving of 5 mm within the first week but thereafter only small and statistically insignificant savings were observed until September 13. On the other hand, from July 12 to 19 and August 16 to 23, the loss of water from the treated plots was greater than from the untreated ones. These small but statistically significant losses resulted in the soil water content of the treated plots approaching that of the checks during these 2 periods (fig 1). In the final period between September 13 and October 25, the loss of water was again greater from the check plots than the sprayed ones, resulting in a saving of 7.5 mm. The total saving for the season was 29 mm. As in 1966, the savings of water due to PMA were restricted to the upper 183 cm of the soil profile.

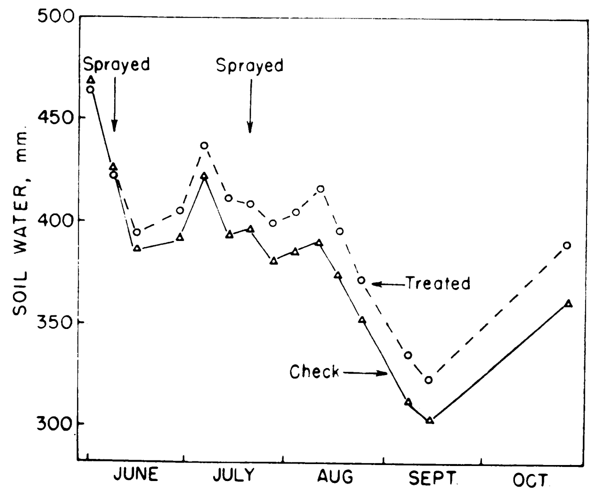

FIG. 1. Water content in the upper 183 cm of the soil throughout the season in sprayed and unsprayed plots.

The soil water content of each 30 cm interval in the profile is not presented, but the data showed that the summer rains occassionally recharged the upper portions of the profile. However, rains were never sufficient to recharge the whole profile as the results in figure 1 indicate. If the entire soil profile had reached field capacity (which does occur during the winter months), then the savings from the use of PMA would increase the stream flow from the forest as a result of the increased leaching and run-off from the treated plots.

Leaf Resistance. The observations of leaf resistance, taken during daylight hours when the level of incoming radiation was similar in both treated and untreated trees, showed that prior to spraying the trees with PMA, there was no difference in the leaf resistance. However, measurements taken soon after spraying on June 8 showed a significant increase in the resistance from 12 seconds in the check needles to 19 seconds in the treated ones. The

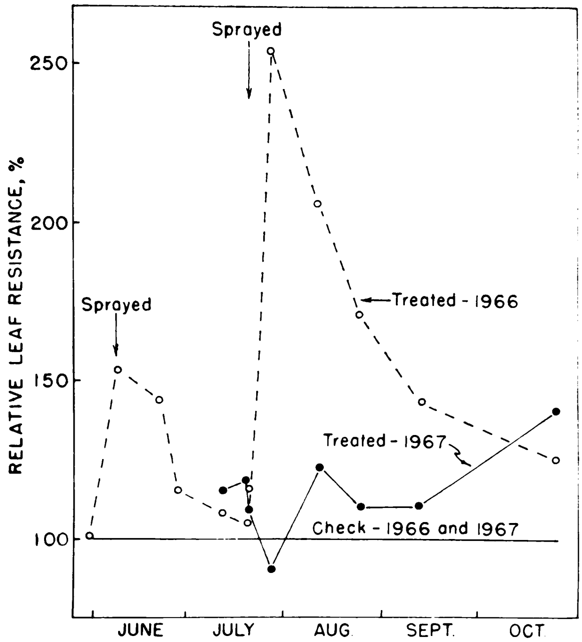

FIG. 2. The relative change in leaf resistance in 1966 and 1967 needles due to spraying with PMA.

effect of PMA soon began to diminish, as the relative values in figure 2 indicate, so that by July 12 a significant difference in the resistances no longer remained. The further treatment of the trees on July 19 increased the resistance in the treated needles to 2.5 times that in the checks for those needles which had been sprayed earlier. In the 1967 needles, which were mere scales in early June, PMA had no effect. With time, the effect of PMA on the 1966 needles declined but was still significant at the conclusion of the observations in late October. The rate of water loss in the 1967 needles was 3 times that in the 1966 needles at emergence in July but decreased with time so that it was only 1.5 times as great by October. There was some indication that the difference between sprayed and unsprayed 1967 needles increased with time as the needles aged and their rate of water loss decreased.

Leaf Water Potential. Measurements of leaf water potential, under the same conditions as those of leaf resistance, were commenced 21 days after the trees were sprayed on June 8. On this date, the water potential of the needles was significantly reduced from 14.2 bars in the check trees to 12.9 bars in the treated trees, but this difference had disappeared by July 12. The further treatment with PMA on July 19 again decreased the potential in the treated trees by 2.3 bars, but this difference gradually decreased (fig 3), as the effect of the chemical on the stomatal aperture decreased, until statistically significant differences could not be established on October 25.

Bole Contraction. Prior to spraying the trees on June 8, there was no significant difference in the contraction of the bole measured with the dendrom-

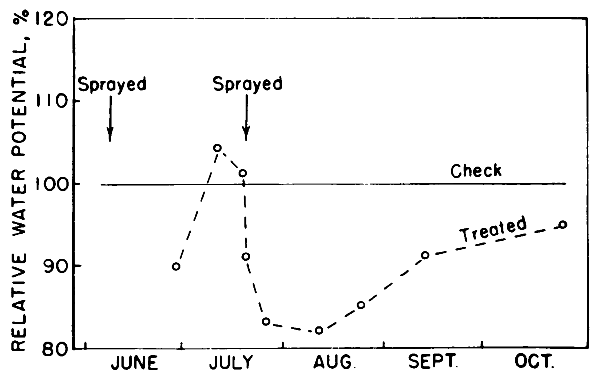

FIG. 3. The relative change in water potential of the needles throughout the season due to spraying with PMA.

eter. After spraying, however, the contraction in the sprayed trees was only 78 microns compared with 114 microns in the unsprayed trees. Although the magnitude of the bole contraction varied with environmental conditions, the difference in bole contraction varied little, and contraction was always about 25 % less in the treated than in the untreated trees for the remainder of the season.

Growth. As in 1966, PMA had no effect on the length and dry weight of the needles. However, radial growth was markedly reduced as shown by figure 4. In the week following spraying on June 8, the daily rate of growth was reduced from 34 microns in the check trees to 22 microns in the treated ones. Significant reductions in growth were recorded on all subsequent occasions until August 24 when further increases in bole diameter were negligible. PMA caused a reduction of almost 40 % in radial growth over the whole season.

A study of the increment cores from the same trees showed that the treated ones had produced

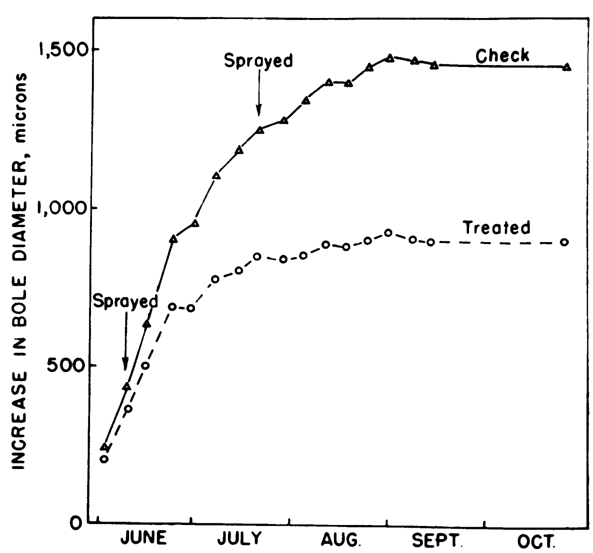

FIG. 4. Increase in bole diameter measured with the dendrometer in sprayed and unsprayed trees throughout the season.

only 53 % of the xylem growth of untreated ones. The spring and summer wood were similarly affected. However, the width of the growth rings in 1965, before treatments were imposed, showed that the trees selected for treatment had, by chance, produced 17 % less growth than those selected as check trees. A comparison of the xylem growth for 1967 with 1965 showed that it was reduced 32 % by treatment. Since the increment growth was found to be less than radial growth measured by the dendrometer (4) and the phloem was also reduced by 10 % in the treated trees, the 2 measures of the effect of the spray on growth are comparable. Since no correlation between the xylem growth of a tree and its water use could be established, we concluded that the difference in water use by treated and untreated trees was not a result of the chance choice of faster growing trees in the check plots but was a result of the treatment imposed.

No damage to the foliage by PMA was observed in the plots, but the number and dry weight of the needles which fell from the treated trees was significantly greater in the period between September 15 and October 25. The number of fallen needles rose from 2300 per m^2 in the check plots to 3950 per m^2 in the treated ones. The dry weights were 149 g and 257 g respectively. However, in the subsequent 6 weeks, only 550 needles per m^2 (34 g dry wt) were collected in the check plots, a number not significantly different from that in the treated plots.

Diurnal Change in Leaf Resistance, Leaf Water Potential and Bole Contraction. For one 24 hour period from 1400 hours on July 26, the changes in radiation, leaf resistance, leaf potential and bole contraction for 2 trees in the forest are presented in figure 5. The rapid changes in light intensity in the tree crowns near sunrise and sunset caused a rapid change in leaf resistance. The closure of the stomata at sunset caused a reduction in the leaf water potential from 12 to 14 bars during the day in the unsprayed trees to a minimum of 2.5 bars near dawn. Similarly, the bole contraction decreased overnight to a minimum at 0800 hours but rose again during the day.

PMA increased the stomatal resistance in the 1966 needles during the daylight hours. The 1967 needles responded to the diurnal fluctuations in light in the same manner as the 1966 needles except that the resistance during daylight was lower and, as previously noted, there was no response to PMA. The partial stomatal closure in the 1966 needles decreased the leaf water potential during the day and the bole diameter contracted less. In all cases,

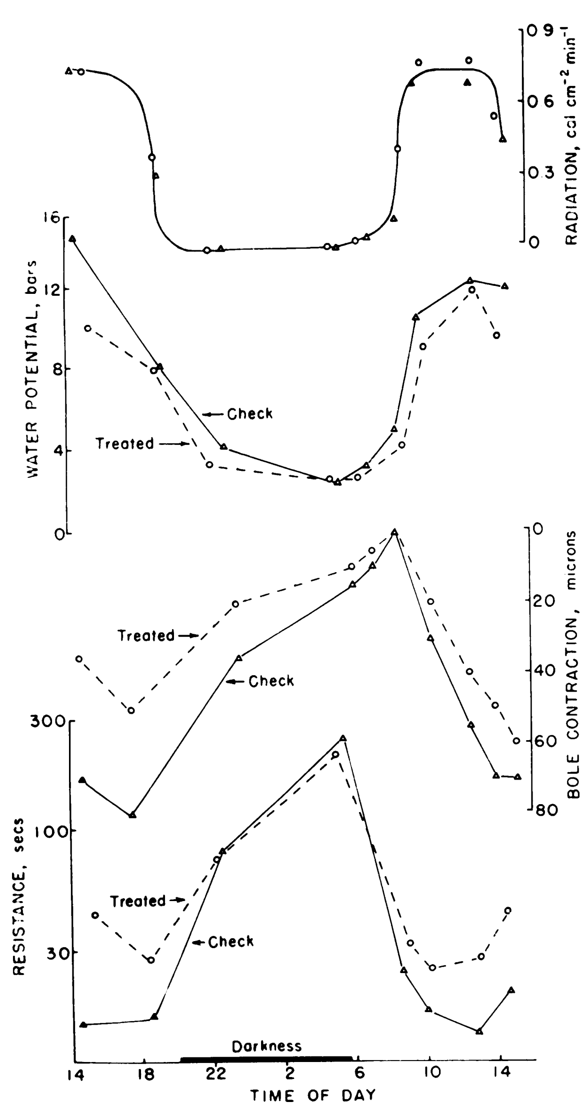

FIG. 5. Diurnal variation in radiation, leaf water potential, bole contraction, and leaf resistance in sprayed and unsprayed trees.

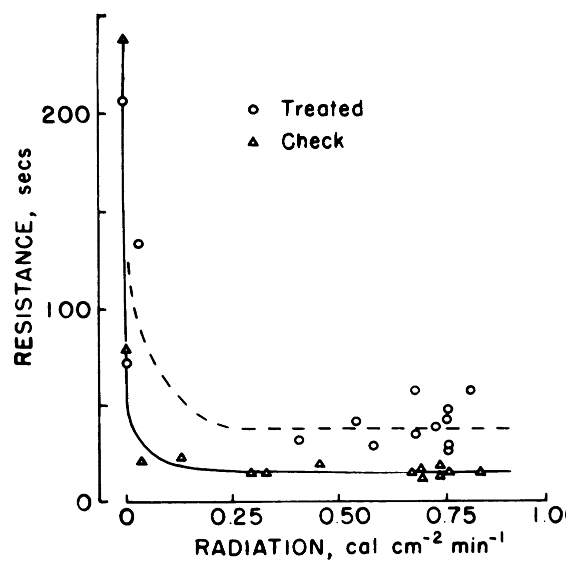

FIG. 6. The effect of radiation on leaf resistance in sprayed and unsprayed needles.

the difference between the treated trees and checks was greatest in the middle of the day and declined overnight until there was no difference at dawn.

The effect of the level of radiation on leaf resistance can be seen more clearly from figure 6. Once the incoming radiation was higher than 0.25 cal cm^{-2} min^{-1}, there was no change in the leaf resistance, but the resistance increased sharply when the incoming radiation decreased below 0.1 cal cm^{-2} min^{-1}. The effect of PMA in partially closing the stomata can also be seen. The 1967 needles responded to the level of incoming radiation in the same manner as the 1966 needles except the curve for both treated and untreated needles was below the lower curve in figure 6. The increased variability in the resistance of the 1966 needles from the sprayed trees presumably represents the variation in coating the needles with PMA since each point represents duplicate samples of only 5 fascicles.

No change in leaf resistance could be detected for leaf water potentials between 5 and 15 bars during that period of the day when the incoming radiation was above 0.25 cal cm^{-2} min^{-1}.

Discussion

The continuation of the experiment commenced in 1966 (15) confirmed the results then obtained that partial closure of the stomata by small quantities of PMA does significantly change the evapotranspiration by the forest. 1967 was a much wetter year than 1966, and the moister soil, plus the further treatment of the trees once the new needles had been produced, resulted in a greater saving. The evapotranspiration between June 1 and October 25 was 9 % less on the plots sprayed with PMA with a maximum saving of 25 % occurring on 3 separate occasions during the summer.

The use of the spray, after the 1967 needles had grown, had a smaller effect than expected. This was evidently because PMA failed to increase the leaf resistance of the new needles. Since water loss from the 1966 needles was only a third as fast as from the newly emerged needles, the further closure of the 1966 needles was relatively ineffective. The reason PMA did not partially close the stomata in the 1967 needles is not known.

The 15 % reduction in bole growth recorded in 1966 cannot now be attributed to the effect of stomatal closure as a comparison of the 1966 and 1965 xylem growth increments revealed no difference in growth. However, there was a definite reduction of approximately 32 % in the radial growth during 1967. Since stomatal closure hinders exchange of carbon dioxide as well as water, some reduction in the photosynthetic capacity of the needles was expected. The results appear to confirm those of Keller (6) who reported that PMA reduced photosynthesis more than transpiration in spruce trees. Nevertheless, it is probable that the reduction in total dry weight in this experiment was not as great as the reduction in radial growth since the needle and the extension growth were unaffected.

In any 1 year about 25 % of the needles in red pine fall to the ground, the peak numbers falling during September and October (figures kindly supplied by G. R. Stephens of The Connecticut Agricultural Experiment Station). In drought years, the number is increased. The increased number and dry weight of needles which fell from the treated trees is probably a result of PMA causing earlier needle senescence. This will affect the growth and transpiration in ensuing years. Alternatively, the increase may be the result of the better plant hydration in the treated trees during 1966, which was a drought year, causing a greater retention of the needles in the treated trees in that year.

The seasonal changes in leaf resistance showed that the effectiveness of PMA in closing stomata began to diminish within 2 weeks. Within 1 month of spraying on June 8, no significant differences in leaf resistance or saving of water could be established. This agrees well with the results obtained in 1966 (15). Zelitch and Waggoner (18) previously reported that the closure usually lasted for at least 2 weeks in tobacco, and Slatyer and Bierhuizen (12) observed a reduction in transpiration for at least 4 weeks in cotton. Davenport (3) reported that 10^{-3} M PMA reduced transpiration in creeping red fescue (*Festuca rubra* S-59) grown in lysimeters for 2 weeks after the first spray and just over 3 weeks from a second spray. Keller (6) in 1 experiment obtained a reduction in transpiration in spruce trees for a period of at least 74 days using 10^{-3} M PMA. In a previous experiment, a second spray with 10^{-3} M PMA had killed the trees. In our case, the further spray in July did not cause any apparent damage to the foliage but did increase stomatal resistance more, and more persistently, in affected needles. Differences in leaf resistance were still significant 9 weeks after spraying although they were much smaller than those observed within 1 week of spraying.

The diurnal changes in leaf resistance showed no evidence of stomatal closure during the afternoon despite a leaf water potential of 15 bars. There are few observations of the effect of the leaf water potential on leaf resistance, but it is concluded from observations of the relative water content of leaves and its effect on the rate of transpiration that the potential at which stomatal closure occurs varies with species and environment (11). Begg *et al.* (1) found that stomata closed in millet grown in high radiation at a leaf water potential of about 20 bars. Since leaf resistance was unchanged when the leaf water potential varied between 5 and 15 bars, water deficit, in itself, evidently does not affect stomatal aperture until a critical tension is reached. Between June and September 1967, the soil water levels were always high (fig 1), and no observations were made when evaporative demand was very great. Under more extreme conditions, stomatal closure could be expected.

The contradiction of the bole shows that the plant uses the water in the phloem and outer xylem vessels to meet the demand created by the lag in absorption by the roots over the loss to the atmosphere. Diurnal variations in the water content and diameter of tree boles have been previously reported (7), but the importance of the bole as a source of water has not been evaluated. The reservoir of water available in the bole of a tree would be expected to allow high transpiration rates to be maintained for longer periods of stress than in crops such as corn or tobacco.

The dependence of stomatal resistance on light intensity resembles that of turnip (5) and cotton (13). Resistance changed surprisingly little when the radiation flux density exceeded 0.25 cal cm^{-2} min^{-1}. This explains the rapid opening and closing of the stomata in the needles in the upper whorls near sunrise and sunset. However, incoming radiation often falls below 0.25 cal cm^{-2} min^{-1} beneath clouds. Thus, partial stomatal closure was observed in the forest during the cloudy afternoon of July 27 (fig 5). Radiation also decreases with penetration into the canopy, and again radiation flux densities below 0.25 cal cm^{-2} min^{-1} are observed even when the sun is shining. Thus, stomata in the lower parts of the canopy should show partial closure. This was observed, but the data are not reported here.

The use of PMA in a forest stand has demonstrated the possibility of manipulating the vegetation to reduce water loss and the feasibility of using small quantities of chemicals to save water. PMA has previously been shown to be the most effective chemical used to date, but the deleterious effect on growth makes the search for new chemicals important.

Acknowledgments

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