The Influence of Environmental Factors on the Sensitivity of Virginia Pine to Ozone

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Contribution No. 666, Department of Plant Pathology, The Pennsylvania Agricultural Experiment Station. Authorized for publication 19 April 1972 as Journal Series Paper No. 4191. Contribution No. 256-72, Center for Air Environment Studies.

We acknowledge the Office of Manpower Development, National Air Pollution Control Administration of the U.S. Public Health Service, for providing Grant No. AP 00436 and Special Fellowship No. 5 F3 AP 33, 925; and the School of Forest Resources for providing space in the Forestry Resources Laboratory.

Accepted for publication 9 September 1972.

ABSTRACT

Virginia pine seedlings were injured more severely when exposed to 25 pphm O_3 for 4 hr at high humidities than when exposed to this dose at low humidities. The percent relative humidity at which plants were maintained before or after exposure did not affect the amount of injury. An inverse relationship was observed between exposure temperature and degree of plant injury. In contrast, a direct correlation was observed between the temperature at which plants were maintained before and after exposure and the amount of O_3 injury. Seedlings kept in the light for 24 hr or longer prior to exposure were protected from injury. Plants maintained in darkness for periods up to 96 hr before exposure were injured. Postexposure extended light periods did not affect symptom development; however, extended dark periods following exposure delayed typical symptom development until the seedlings were placed in the light.

Phytopathology 63:371-376

Additional key words: air pollution, phytotoxicants, trees.

It is well known that physical environmental factors such as heat, moisture, and light affect the response of plants to air pollutants. However, most of the information currently available was derived from studies of herbaceous plants or was concerned with pollutants other than ozone (O_3). Relatively little is known of the effects of physical environmental factors on the response of woody plants to O_3. Costonis & Sinclair (3) recently reported that eastern white pine was severely injured when exposed to O_3 at a high temperature, in mist, and maintained at a high temperature following exposure. Nonconiferous species, such as tobacco, when maintained at 5 C overnight were less sensitive to O_3 than when they were kept at 25 C; also, a 5-C difference during a 2-week cultural period resulted in less injury on the plants grown at the lower temperature (10). Heck et al. (7) reported an inverse relationship between exposure temperature and amount of O_3 injury on tobacco and pinto bean plants. Otto & Daines (12) found a direct correlation between O_3 injury on tobacco and pinto bean and exposure humidity.

Wilhour (13) recently reported a direct relationship between O_3 injury on white ash and the temperature at which plants were maintained before or after exposure. He also reported a direct relationship between O_3 injury and relative humidity prior to exposure. In contrast, an inverse relationship was noted between exposure temperature and amount of injury. There was no significant correlation between exposure or postexposure humidity levels and amount of injury.

Dugger et al. (5) reported that 48-72 hr of darkness prior to exposure protected bean plants from injury. In contrast, Menser et al. (10) found that tobacco plants kept in the dark for 14-66 hr prior to exposure to O_3 were severely injured, and that a 22-hr extended photoperiod immediately before exposure gave nearly complete protection. There have been no reports concerning the effect of long dark or light periods on conifer sensitivity to O_3.

Eastern white pine has been reported to be very susceptible to O_3 (1, 2, 3), and also to be relatively resistant (14). Varying results such as these may be due in part to different workers using different environmental regimes before, during, or after exposure of plants, or to inherent genetic differences in susceptibility. If environmental factors were determined and standardized before, during, and after exposure, more meaningful comparisons could be made among reports from various workers. Also, such data may be of value in predicting plant losses from O_3 and in the elucidation of mechanisms of injury.

The influence of environmental factors must also be considered when formulating air quality standards for vegetation with respect to O_3 (8). Small changes in relative humidity or temperature may greatly influence the threshold levels of O_3 needed to cause plant injury. For example, the amount of O_3 needed to cause injury on a certain plant species growing in less humid areas of western USA may be considerably more than that needed to injure the same species growing in a humid area of eastern USA.

Although Virginia pine has been reported to be susceptible to O_3 (4, 14), the influence of environmental factors on its sensitivity has not been reported. This study was initiated to determine the influence of relative humidity and temperature on the sensitivity of primary and secondary needles of Virginia pine to O_3 and to determine the influence of long periods of light and dark on sensitivity.

MATERIALS AND METHODS.—Juvenile plants, less than 1 year old, with primary needles were grown from seed and maintained in greenhouses; 3-year-old seedlings with secondary needles were grown outdoors. All plants were potted in a 3:1 peat:perlite mixture and were watered regularly. No fertilizer or pesticides were applied. The foliage of all plants was examined prior to exposure. Any markings which could be confused with those resulting from exposure to O_3 were recorded.

Ozone was generated by the passing of pure oxygen through a commercial ozonator that utilized an ultraviolet light. The gas was then transferred to the exposure chamber through Teflon tubing, where it mixed with the charcoal-filtered air of the chamber.

The exposure chamber was a modified version of a commerically available chamber (Environmental Growth Chamber Co., Chagrin Falls, Ohio) and has been described elsewhere (15). During exposure, O_3 levels were constantly monitored with two Mast O_3 meters (Mast Development Co., Davenport, Iowa) connected to a strip chart recorder. Oxidant values of the Mast meters were compared to those obtained using the neutral buffered-potassium iodide method. Meter efficiencies ranged from 80 to 85%. Continuous and intermittent temperature measurements were made during exposures, using copper-constantan thermocouples connected to a 24-point recorder. Relative humidity was monitored using lithium chloride probes and a single pen strip chart recorder.

Unless otherwise noted, O_3 exposures were conducted at 25 pphm for 4 hr at a light intensity of 2,400 ft-c. In relative humidity studies, temperature was maintained at a constant 24 C; relative humidity was held at a constant 75% in all temperature studies, and a minimum of 20 plants were used in each experiment.

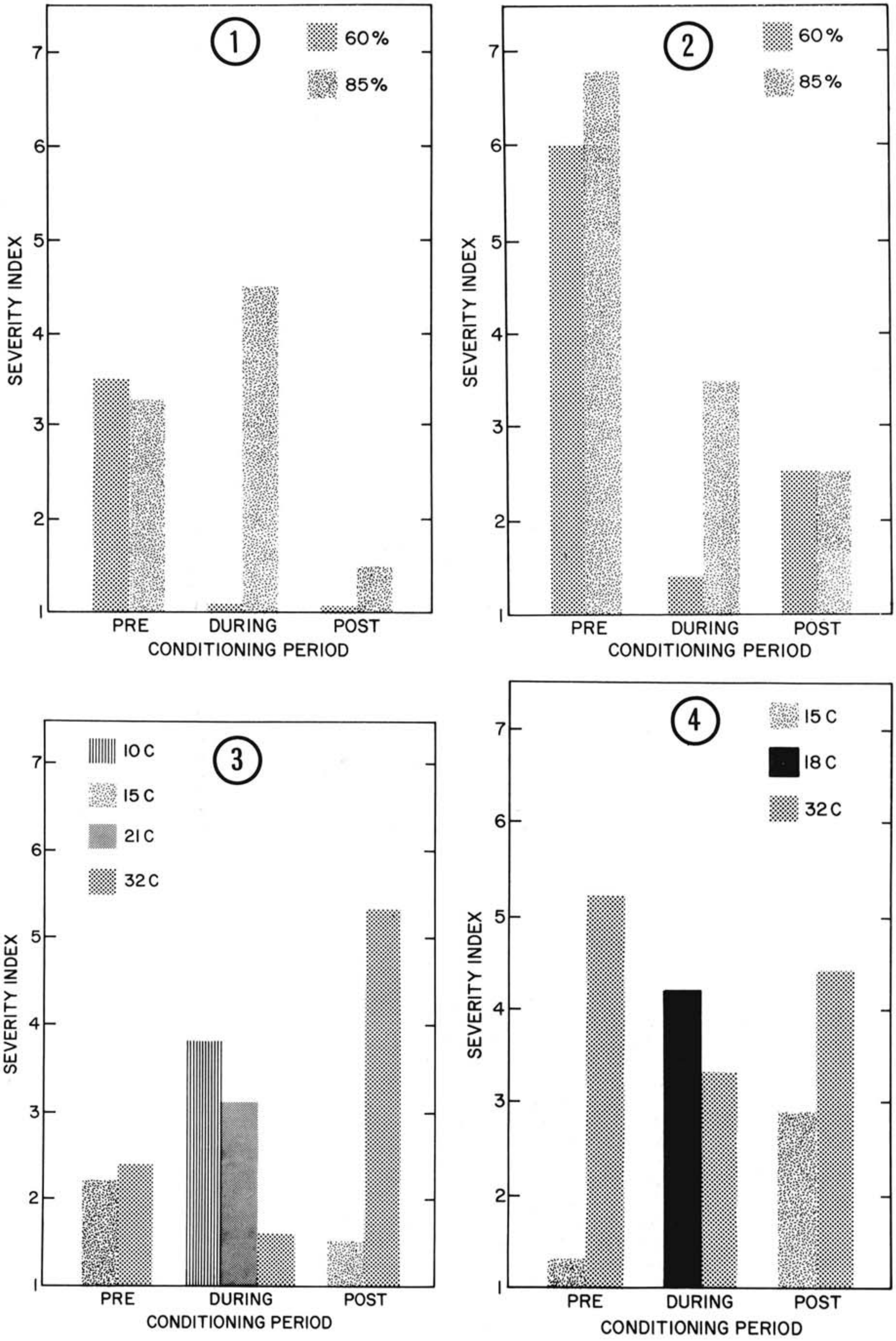
In the pre-exposure studies, plants were maintained for 1 week at the conditioning environmental regimes in Model M-3 Environmental Growth Chambers. In the humidity studies, one chamber was kept at a constant 60% and the other at 85%; in the temperature studies, one chamber was maintained at 15 C and the other at 32 C. The influence of exposure environmental conditions was studied by maintenance of relative humidity at 60 or 85% or by temperature variations from 10 to 32 C. In the postexposure humidity studies, seedlings were maintained for 1 week at 60 or 85% relative humidity. Plants were kept for 1 week at 15 or 32 C in the postexposure temperature studies.

Symptoms were evaluated on individual needles 1 week after the exposure using a severity index of 1-7. A value of "1" = no injury, "2" = a very slight chlorotic mottle, "3" = a more severe mottle, and so forth to "7", which indicated tissue necrosis and collapse.

An average severity index was calculated for each plant. It was based on all primary needles of juvenile plants and on a sample of 40 secondary needles taken from the first flush of current growth on the terminal branch of each 3-year-old seedling.

To study the influence of light and dark periods on the response of Virginia pine to O_3, 3-year-old seedlings were subjected to periods of 12, 24, 28, 72, and 96 hr of light or darkness prior to or after exposure. Four plants were used for each treatment, and the experiment was replicated twice. During the

Fig. 1-4. 1) Influence of pre-, during, and postexposure relative humidity regimes of 60 and 85% on sensitivity of primary needles of Virginia pine exposed to 25 pphm O_3 for 4 hr. **2)** Influence of pre-, during, and postexposure relative humidity regimes of 60 and 85% on sensitivity of secondary needles on 3-year-old Virginia pine plants exposed to 25 pphm O_3 for 4 hr. **3)** Influence of pre-, during, and postexposure temperature regimes of 10, 15, 21, and 32 C on sensitivity of primary needles exposed to 25 pphm O_3 for 4 hr. **4)** Influence of pre-, during, and postexposure temperature regimes of 15, 18, or 32 C on sensitivity of secondary needles of 3-year-old Virginia pine plants exposed to 25 pphm O_3 for 4 hr.



1
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pre- and postexposure period, temperature was maintained at 24 C during the "day" and 18 C at "night"; relative humidity was maintained at 70% during the day and 60% at night. A day-length of 12 hr, from 6 AM to 6 PM, was used. The plants were exposed to 50 pphm O_3 for 1.5 hr; a concentration-time combination was selected so that the plants would be exposed to a light period of minimum length during exposure. Plants that received the dark treatment were placed in the light for 20 min prior to exposure to enhance stomatal opening.

In previous studies, it was noted that symptoms were usually more pronounced on the upward-facing needle surfaces. To determine whether this localization of symptoms on the upper needle surfaces was inherently controlled or was due to artificial factors of the exposure procedure, potted seedlings were placed horizontally on the plant bed of the exposure chamber during fumigation. These plants were kept in a normal upright position before and after exposure to O_3.

In order to determine whether the overhead light or downward flow of air might be responsible for the localization of symptoms, a transparent mylar "shield" was supported over Virginia pine seedlings prior to exposure. The shield was set up so that O_3 would not impinge directly on the upper surfaces of leaves, but rather would come in contact only with the leaves from the sides of the plants.

Analyses of variance were performed on the data. Probabilities greater than 95% were recorded; probabilities less than 95% were considered nonsignificant.

RESULTS.–Figure 1 illustrates the influence of relative humidity on sensitivity before, during, and after exposure of 60 juvenile plants with primary needles to 25 pphm O_3 for 4 hr. These three studies were conducted using plants of slightly different ages. Therefore, comparisons should be made only between the high and low environmental factors within each study, rather than among the three studies.

There was no significant difference between the amount of injury on those plants preconditioned for 1 week at 85% relative humidity prior to exposure compared to those plants kept at 60%. Plants exposed at 85% humidity had significantly higher severity ratings than did those exposed at 60%. After exposure, plants maintained at the higher humidity did not have significantly more injury than those kept at the lower humidities.

The influence of relative humidity on the sensitivity of 60 3-year-old plants exposed to 25 pphm O_3 for 4 hr is shown in Fig. 2. Ten plants preconditioned for 1 week at 85% relative humidity prior to exposure had significantly higher severity indices than did those plants maintained at 60%. Plants exposed at 85% relative humidity had significantly more injury than did those exposed at the lower humidity regime. Three-year-old plants maintained at 85% relative humidity after exposure had approximately the same amount of injury as did those maintained at 60%.

The influence of temperature on the response of 70 juvenile plants to 25 pphm O_3 is shown in Fig. 3. There was no significant difference in the severity of symptoms between the plants conditioned at 15 or 32 C prior to fumigation. However, during exposure, seedlings exposed at 10 and 21 C had significantly higher severity ratings than did those plants exposed at 32 C. Also, plants maintained at 32 C for 1 week after exposure were injured significantly more than were those plants kept at 15 C.

Figure 4 illustrates the influence of temperature on the sensitivity of 60 3-year-old seedlings exposed to 25 pphm O_3 for 4 hr. Plants preconditioned for 1 week at 32 C were significantly more sensitive than were those kept at 15 C. An inverse relationship was noted between exposure temperature and plant injury. This relationship was not significant. Plants maintained at 32 C after exposure had significantly higher severity ratings than did those kept at 15 C.

A second study utilizing 50 seedlings revealed a significant inverse relationship between exposure temperature and amount of O_3 injury on 3-year-old plants (Fig. 5). Plants exposed at 10 or 15 C had significantly more injury than did those exposed at 32 C. Other comparisons were nonsignificant.

Seedlings kept in the light for 24 hr or longer

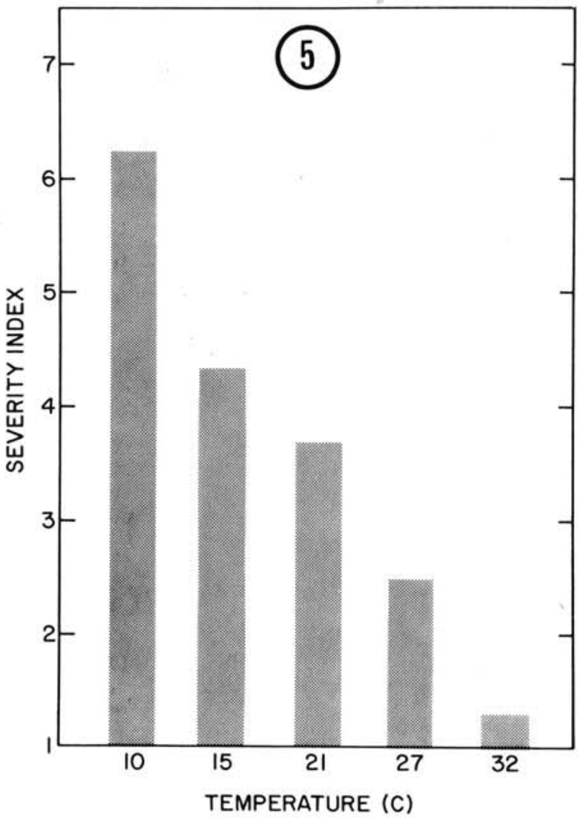

Fig. 5. Influence of exposure temperature on sensitivity of secondary needles of 3-year-old Virginia pine plants exposed to 50 pphm O_3 for 1.5 hr.

prior to exposure were protected from injury. In contrast, plants maintained in the dark for periods up to 96 hr prior to exposure were injured when exposed to O_3.

There was little difference in final symptom development between plants maintained in a postexposure dark period in contrast to those kept in a postexposure light period. However, symptoms developed much more rapidly on those plants kept in a postexposure light period; symptom development was delayed on plants placed in the dark after fumigation. Plants that had received a dark treatment after exposure possessed faint symptoms of the water-soaked type. When these plants were placed in the light, the water-soaked symptoms developed into a typical symptom.

Symptoms were usually most severe on the needle surfaces facing the lights. Plants exposed in a horizontal position had most severe symptoms on the needle surfaces facing the light during exposure, rather than those needle surfaces normally facing upward. Plants exposed under the mylar shield also had most severe symptoms on the leaf surfaces facing the lights in the ceiling of the exposure chamber.

DISCUSSION.–The response of Virginia pine seedlings to O_3 was not significantly influenced by the humidity regime in which they were conditioned prior to exposure. This is in contrast to results for white ash (13), where more injury occurred in plants preconditioned at a higher humidity. This difference may be due to inherent genetic differences in susceptibility between the two species.

Virginia pine seedlings conditioned at higher temperatures prior to exposure were more severely injured than those maintained at lower temperatures. These results are similar to those reported for white ash (13) and tobacco (10). The inverse relationship of exposure temperature and amount of injury agrees with reports using pinto bean and tobacco (7) and white ash (13). However, Costonis & Sinclair (3) reported that the amount of O_3 injury on eastern white pine was favored by high exposure temperature. Virginia pine plants maintained at high temperatures after exposure were more severely injured than those kept at lower temperatures, similar to results of studies using eastern white pine (3) and white ash (13).

Although preconditioning plants at higher temperatures would result in their having wide stomatal apertures (11) at the time of exposure, it is possible that the influence of temperature on O_3 sensitivity may be more related to subtle physiological effects.

The direct relationship between amount of O_3 injury on Virginia pine needles and exposure humidity was similar to that reported for tobacco and pinto bean (12). The degree of injury on the latter species was directly correlated with increasing size of stomatal apertures, as the level of humidity increased. Since Hodges (9) directly correlated the degree of stomatal opening of various conifer species with increasing humidity levels, it is possible the stomatal opening is also correlated to injury on Virginia pine needles exposed at various humidities.

Variations in conifer leaf water potential have also been correlated with stomatal movement (9). As the amount of leaf moisture changes, the concentrations of solutes including carbohydrates, in the cells may also change. This change may influence the solubility of O_3 or its oxidized products, just as Hodges (9) reported changes in leaf water potential may affect CO_2 diffusion within the leaf.

Within limits, temperature is considered to be directly related to the degree of stomatal opening (11). However, exposure temperature was inversely related to amount of injury. Apparently, degree of stomatal opening is not a factor here. A possible explanation for increased injury to plants exposed at low temperatures involves the solubility of O_3 in the leaf tissues. The solubility of O_3 in water increases with decreasing temperature (6). Thus, increased injury at lower exposure temperatures may be correlated more closely to O_3 solubility in the leaf than to stomatal opening.

The humidity regime at which plants were maintained after exposure of both Virginia pine and white ash (13) was not significantly related to injury. Apparently, the injury process was not influenced by these humidity levels following exposure.

In contrast, the postexposure temperature regime affected the development of symptoms. These temperature influences are not likely to be directly related to stomatal opening, but are probably related to subtle changes in plant metabolism, which in some manner affect its sensitivity to O_3.

If these results are extrapolated to field conditions, the most severe O_3 injury on conifer foliage during the growing season would occur after several days of warm weather, followed by a cool, humid period with high O_3 levels, after which warm weather again prevailed. However, during a single day, the highest O_3 concentrations usually occur in early afternoon, a time when temperatures are often at their daily high and humidities are at a daily low. Consequently, during periods of daily maximum pollutant levels, environmental conditions would often be relatively unfavorable for symptom development.

Plants maintained in light 24 hr or longer prior to exposure were completely protected from injury, whereas those kept in the dark for long periods were severely injured. Thus, it appears that Virginia pine responds to light more like tobacco (10) than pinto bean (5). Plants kept in long light periods following exposure showed typical symptoms, whereas conditioning of plants in long dark periods after exposure only delayed development of typical symptoms. The latter results point out the importance of light in symptom development in plants exposed to O_3.

Symptoms induced on Virginia pine by O_3 were previously described (4, 14). Both abaxial and adaxial faces were sensitive, but the most severe symptoms were on those needle surfaces facing the overhead lights of the exposure chamber. This again emphasizes the importance of light in symptom development.

Chlorotic mottle and tip or needle necrosis were the only symptoms that developed under the variety of environmental conditions employed. These symptoms have been described for other conifers exposed to O_3 (1, 3, 14), and are likely to be the most common ones produced on conifer foliage exposed to O_3.

Both primary and secondary needles responded similarly to conditioning with various temperature and humidity regimes. Thus, young plants with primary needles could be used to study the influence of environmental factors on the response of conifer seedlings to O_3. These small plants require much less space and are easier to handle than the larger seedlings with secondary needles. Also, they can be grown from seed at any time of the year.

In summary, the results of this study indicate that environmental parameters such as relative humidity and temperature influence the response of Virginia pine seedlings to O_3. In many ways the results were similar to those reported from comparable studies using broadleaved plants. Data from this study further suggest that laboratory exposure of plants to O_3 should be conducted under carefully controlled and standardized relative humidity and temperature regimes. This would allow a more meaningful comparison of the results of various investigators.

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