J. AMER. SOC. HORT. SCI. 110(1):106–108. 1985.

Sensitivity of Satsuma Mandarin to Ozone as Related to Stomatal Function Indicated by Transpiration Rate, Change of Stem Diameter, and Leaf Temperature

Jiro Matsushima and Keizo Yonemori
Faculty of Agriculture, Mie University, Tsu, Mie, Japan 514

Kenzo Iwao
Central Technical Research Laboratory of Chūbu Electric Power Co., Ōdaka, Midoriku, Nagoya, Japan 459

Additional index words. air pollution, stomata, *Citrus unshiu*

Abstract. **Transpiration rate, stem diameter, and leaf temperature of satsuma mandarin (*Citrus unshiu* Marc.) were found to be sensitive indicators of stomatal response to ozone exposure. Time of reaction of each to 1.2 ppm ozone was 3 min. Stem diameter showed marked oscillation and leaf temperature slight oscillation, when ozone was applied. Correlation coefficiennt between ozone concentration and transpiration rate at the equilibrium status after the beginning of exposure was $r = 0.718$, and increasing the concentration of ozone decreased stomata size. Thus, it was determined that low sensitivity of satsuma mandarin to ozone is due to rapid stomatal closure by ozone.**

Satsuma mandarins generally are tolerant to many agricultural chemicals, various salts, and air pollutants; but ethylene is an exception (6). Mechanisms for this tolerance may be suggested: that is, leaves absorb few harmful substances because of stomatal closure in response to those substances, or leaves decompose the absorbed substances biochemically.

Since many substances are absorbed through stomata, the function of stomata as related to the mechanism of tolerance in satsuma mandarin was investigated in this study. Stomatal reaction is difficult to examine during exposure treatment, and indirect indicators of stomatal function would be desirable.

Correlation between stomatal function and transpiration, leaf thickness, stem diameter, and fruit diameter have been reported by many researchers, including Kadoya et al. (5), Burrs et al. (1, 2), Iwao et al. (4). Furthermore, Sekiyama et al. (9, 10) showed that oscillation occurred in leaf thickness and stem diameter as a result of exposure of bean and sunflower to ozone. The purpose of this study was to confirm the sensitivity of transpiration, stem diameter, and leaf temperature changes following stomatal function change in satsuma mandarin, and to test the idea that the resistance to ozone is related to a rapid stomatal reaction.

Materials and Methods

Three-year-old satsuma mandarin trees ('Hayashi') were potted in 30-cm diameter containers. All plants were treated in a growth chamber, 1 m W × 0.8 m D × 1 m H, maintained at 30°C, 75% RH, and 0.24 cal $cm^{-2}min^{-1}$ light intensity.

Transpiration rate was measured by the rate of decrease in the whole weight of container and plant. The container was wrapped by vinyl film to eliminate evaporation from the surface of container and soil. The total weight was transduced into DC voltage (0~2V) by a "load cell", in which a strain-gauge type weight/voltage transducer was set, and then weight was recorded automatically.

The change of stem diameter, shrinkage or swelling, was transduced into DC voltage ($-2\sim+2$ V/$-0.4\sim+0.4$ mm) by a displacement detector. This device was an electrical bridge formed by strain-gauges similar to those reported previously (4) and was attached to the stem, and the amount of change was determined automatically. Leaf temperature was measured by a recording thermister which was an earring shape, and the size of the probe was 0.5 mm in diameter.

In order to obtain the relationship between leaf area (i.e., the transpiration area) and the amount of change of stem diameter, and also the water potential in the twig, leaves of satsuma mandarin tree were reduced in number successively by hourly defoliation. The decrease in stem diameter per hour and the transpiration rate then were measured as leaf number decreased. At the same time, the water potential of the twig with 4–5 leaves in spring flush also was measured by a pressure chamber (Wescor Co.) as leaf number decreased.

Concentration of ozone was determined by a recording ozone meter. Exposure concentrations were between 0.05 and 1.2 ppm. Durations of exposure were 30 min.~15 hr with various combinations, for example 0.25 ppm × 2.5 hr, 1.2 ppm × 1 hr. Measurements were done on 3 trees in each treatment. As the 3 measurements were consistent, typical results from the charts of recorder are demonstrated in figures.

Results

It was necessary to certify that transpiration rate, stem diameter and leaf temperature, indicators of water status as related to stomatal function, are constant when environmental conditions are constant. Figure 1 demonstrates that these 3 reactions were almost constant under controlled condition of 30°C, 75% RH, and 0.24 cal $cm^{-2}min^{-1}$ light intensity. Stem diameter was the most variable, but was still usable for this research.

Tests also were done to certify that stem diameter and transpiration rate response to water status was related to stomatal aperture in our experiment conditions. A satsuma mandarin tree was defoliated hourly to decrease the transpiration area. Water

Received for publication 5 Mar. 1984. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked *advertisement* solely to indicate this fact.

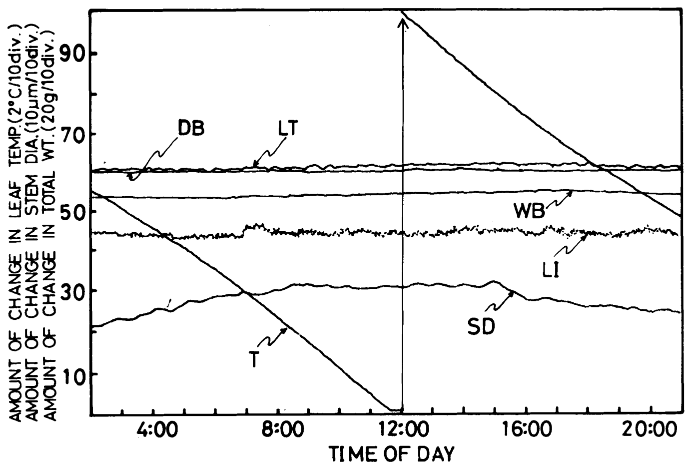

Fig. 1. Time course of change amounts of leaf temperature, stem diameter, and total weight of tree and container in satsuma mandarin. LT:leaf temperature, SD:stem diameter, T:total weight of tree and container (change amount of this means transpiration amount.), LI:light intensity (0.24 cal $cm^{-2}min^{-1}$), DB:dry bulb thermometer (30°C), WB:wet bulb thermometer (27°C)

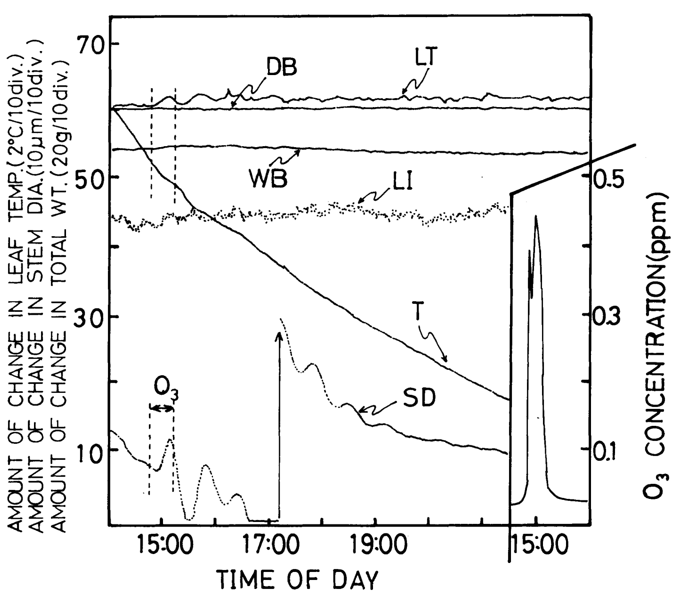

Fig. 2. Effect of ozone in 0.45 ppm on leaf temperature, stem diameter, and total weight of tree and container in satsuma mandarin. Marks in the figure and notes are same as Fig. 1.

potential thus increased, and stem diameter also increased along with a decrease in leaf number. There were very close relationships among leaf number, transpiration rate, water potential and stem diameter. For example, the correlation coefficient between water potential and decreased stem diameter was $r = 0.993$. From these results, it was recognized that transpiration rate and change of stem diameter reflected stomatal aperture when leaf number was constant.

Effects of various concentrations and exposure durations of ozone on transpiration rate, stem diameter, and leaf temperature were measured. Transpiration rate was 22.0 g/hr/tree before exposure, but decresed to 10.0 g/hr/tree by 0.45 ppm ozone for 30 min, as shown in Fig. 2. Stem diameter was increased and showed oscillation, similar to that from light also observed in this experiment. Leaf temperature increased just after the beginning of the exposure. These reactons apparently related to stomatal closure by ozone, and response time was very short.

To confirm response time further, the effect of a high ozone concentration (1.2 ppm) was measured by recording at 20 cm/hr chart speed. Leaf temperature rose 3 min after the beginning of exposure and transpiration rate, and stem diameter also responded in 3 min, as shown in Fig. 3. The time of closing of stomata by ozone thus was less than 3 min, perhaps beginning soon as exposure was initiated.

Transpiration rate responded to ozone rapidly in various concentrations. The correlation coefficient between concentrations and the relative values of transpiration rate (i.e., the percentage compared with that before exposure) was high, $r = -0.717$ as shown in Fig. 4. As these transpiration rates were at equilibrium status soon after the beginning of exposure, these measurements

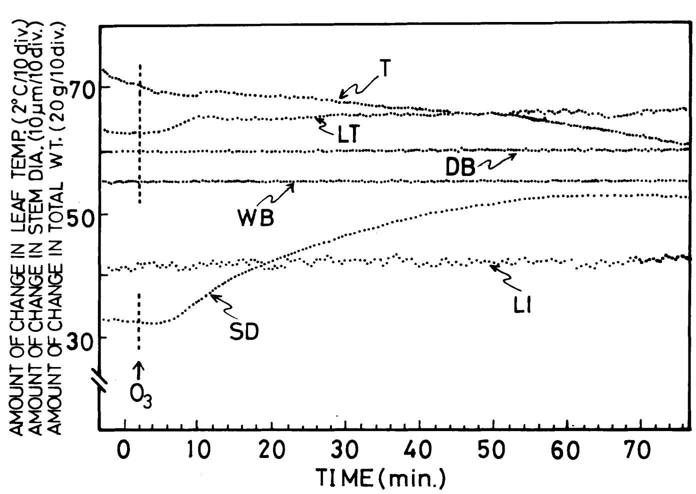

Fig. 3. Effect of ozone in 1.2 ppm on leaf temperature, stem diameter, and total weight of tree and container in satsuma mandarin. Marks in the figure and notes are same as Fig. 1.

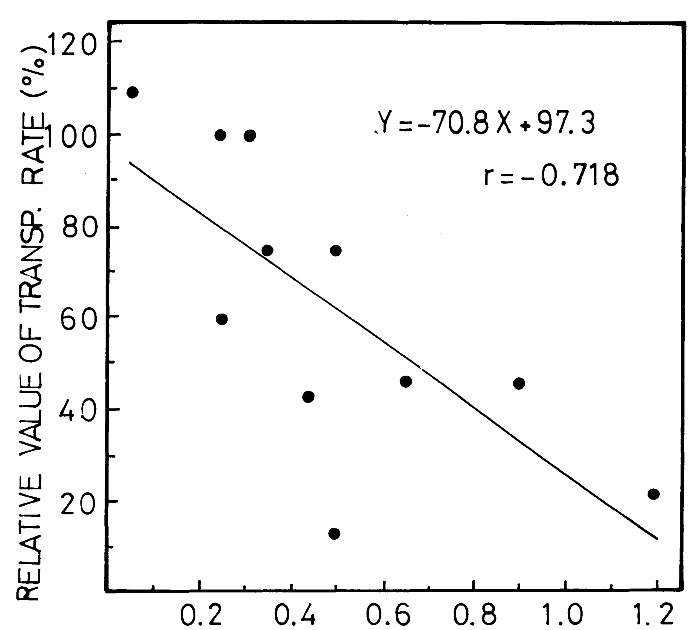

Fig. 4. Relationship between ozone concentration and the relative value of transpiration rate just after the beginning of exposure compared with that before exposure.

were indifferent to further exposures. According to the regression curve, stomata tended to close just after initial exposure at any concentration. Moreover, the higher the concentration, the greater the degree of closure, with maximum closure occurring at about 1.2 ppm. The relationship between ozone concentration and increased stem diameter was not consistent, however. Stem diameters differ from a tree, and the changes might be affected by the diameter as well as by ozone.

At transpiration equilibrium following exposure, the relationship between dose (ppm·hour) and relative value of transpiration rate was lower than the above results ($r = -0.50$).

According to these results, it was assumed that transpiration rate decreased both with concentration and dose, but the more important factor to stomatal closure may be the concentration rather than the dose.

Discussion

From our research, it was assumed that the rate of transpiration, change in stem diameter, and leaf temperature were good indicators for changes in stomatal apertures in satsuma mandarin. The stomata were sensitive to environmental conditions of light and ozone and responded within 3 min to ozone. This response was very clear, and the higher the concentration of ozone, the less was the stomatal aperture. Consequently, one reason for tolerance in satsuma mandarin to various pollutants is due to rapid, almost complete, closure of stomata.

Matsushima and Yonemori reported previously (7) that diffusive resistance indicated the degree of stomatal aperture, and it was increased markedly in 0.2 and 0.5 ppm ethylene. Also, the content of free abscisic acid in seedlings of *Citrus natsudaidai* was increased by ethylene. The diffusive resistance decreased again from the maximum value at 24 hr in spite of ethylene exposure, however, and abscisic acid content showed the same tendency. Matsushima also recognized (6) that satsuma mandarin dropped its leaves entirely after a continuous 48 hr exposure. This severe drop probably is related to the reopening of stomata after 24 hr. It will be necessary to determine if reopening of stomata also may occur in the presence of ozone.

Guard cells are affected by various environmental conditions. Shertz et al. (11) reported that stomata of grapevine were opened by ozone and sulfur dioxide, and the higher the concentration of ozone, the greater the stomatal aperture. In contrast, Rosen et al. (8) showed that stomatal resistance of grapevine increased 30% by ozone in 0.5 ppm, and increased 190% by 0.5 ppm sulfur dioxide. Elkiey et al. (3) reported that diffusive resistance of petunia was increased by 0.4 ppm ozone at low relative humidity (50%) but did not change at high relative humidity (90%). According to the regression curve, stomata would close at any concentration of ozone, but it was not detected below 0.2 ppm in this study. Further study is needed to determine the threshold concentration and its effects on stomatal function in various environmental conditions, and to confirm whether stomata would reopen during relatively long ozone exposure, as previously reported for ethylene.

Literature Cited

1. Burrs, H.D. and B. Klepper. 1968. Cyclic variations in plant properties under constant environmental conditions. Physiol. Plant. 21:711–730.
2. Burrs, H.D. 1971. Cyclic variations in stomatal aperture, transpiration, and leaf water potential under constant environmental conditions. Ann. Rev. Plant Physiol. 22:223–236.
3. Elkiey, T. and D.P. Ormrod. 1979. Leaf diffusive resistance responses of three petunia cultivars to ozone and/or sulfur dioxide. J. Air Pollut. Cont. Assn. 29:622–625.
4. Iwao, K. and K. Matsuda. 1981. Electrostatic capacitance change in plant stem and the relationship between plant physiological action and environmental factor including soil water contents. Res. Assn. of Soil Physics, Japan. 43:2–13.
5. Kadoya, K., K. Kameda, S. Chikaizumi, and K. Matsumoto. 1975. Studies on the hydrophysiological rhythms of citrus trees. I. Cyclic fluctuations of leaf thickness and stem diameter of natsudaidai seedlings. J. Japan. Soc. Hort. Sci. 44:260–264.
6. Matsushima, J. 1977. Sensitivities of plants to ethylene and nitrogen dioxide, and characteristic change in fine structure of the cell. Proc. 4th. Intl. Clean Air Congr. p. 112–115.
7. Matsushima, J. and K. Yonemori. 1981. Effect of ethylene on fruit drop, growth, photosynthesis, diffusive resistance and abscisic acid of citrus trees. Proc. Intl. Soc. Citriculture. 1:304–307.
8. Rosen, P.M., R.C. Musselman, and W.J. Kender. 1978. Relationship of stomatal resistance to sulfur dioxide and ozone injury in grapevines. Sci. Hort. 8:137–142.
9. Sekiyama, T., K. Okabe, K. Iwao, and Y. Fujino. 1978. Studies on measurement and transaction of bio-information in plants. (1) Continuous measurement of plant growth and electrical characteristics of leaves. Bioenviron. Lab., Ctr. Res. Inst. Elect. Power Indu. Rpt. Japan. No. 478005.
10. Sekiyama, T., K. Okabe, H. Hanyu, K. Iwao, Y. Fujino, and K. Noguchi. 1980. Studies on measurement and transaction of bio-information in plants. (2) Seasonal behavior of stem diameter and dynamic behavior of cycles of leaf temperature and electrical characteristics of leaves. Bioenviron. Lab., Ctr. Res. Inst. Elect. Power Indu. Rpt. Japan. No. 479011.
11. Shertz, R.D., W.J. Kender, and R.C. Musselman. 1980. Effects of ozone and sulfur dioxide on grapevine. Sci. Hort. 13:37–46.