Proceedings of the
AMERICAN SOCIETY FOR HORTICULTURAL SCIENCE
1958 71:320-325

# Effect of Air-Borne Reaction Products of Ozone and 1-N-Hexene Vapor (Synthetic Smog) on Growth of Avocado Seedlings[1]

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Air pollutants cause extensive damage to vegetation in many areas of the United States each year. One class of these pollutants, which cause a characteristic bronzing, silvering and necrosis on leafy vegetables, ornamentals and field crops is identified by the term "oxidants" (3, 6, 7). The toxicants responsible for this specific type of plant damage have been shown to be the reaction products resulting from the oxidation of the hydrocarbons present in refined petroleum and oxidants such as ozone and oxides of nitrogen in sunlight (6).

Growth of tomato plants was reduced significantly by fumigation with synthetic smog produced by combining ozone (0.1 ppm) with vapors of 1-N-hexene (5). These reaction products produced anatomical changes in oat leaf tissue (1) arid greatly reduced the chlorophyll content of duck weed *(Lemna minor)* plants (2). Naturally polluted atmosphere retarded growth of alfalfa, sugar beet, endive, and tomato plants, compared with plants grown in air filtered through activated carbon (4). However, fumigation of Mexican avocado seedlings for six months with the reaction products of ozone and gasoline vapor failed to produce leaf injury or appreciable growth suppression (9).

The purpose of this paper is to report the effect of fumigation with the reaction products of ozone and 1-N-hexene vapor (synthetic smog) upon the growth and metabolism of Zutano avocado seedlings.

## MATERIALS AND METHODS

The fumigation spaces used in this experiment were six-foot glass cubes glazed on five sides, exposed to natural lighting of the out-of-doors. Air was introduced into the fumigation spaces at measured rates and was cleansed by passing through activated carbon. The rate of air How, determined by the rate of introduction of new air into the system, was adjusted for one change a minute. Fumigations were alternated and the plants were interchanged daily between the two glass chambers to reduce the effect of uncontrolled aspects of environment.

The 1-N-hexene was oxidized by mixing known amounts of ozone, 3.5 nil per minute, and 1-N-hexene vapor, 30 ml per minute, in a glass reaction tube, the open end of which was fixed within the fumigation space. Reaction products of these two gases (synthetic smog) were released continuously in the treatment chamber for seven hours a day, five days a week. The identity of the toxicant or toxicants produced during the oxidation of unsaturated hydrocarbons is unknown, but plant injury caused during ill is

reaction process closely resembles injury from natural smog, hence the generalized term "synthetic smog" which was introduced by previous workers.

Severe leaf injury was produced on the primary leaves of 14-day old pinto bean plants placed in the treatment chamber daily as indicators, but no injury was detected on the bean plants which were placed in the control chamber. The injury to the bean leaves was unlike that produced by ozone but resembled the silvering, bronzing and tissue collapse which has been reported as smog or oxidized hydrocarbon injury.

The average daily oxidant concentration, was 0.17 ppm (parts per million) as determined by the release of iodine from Sorenson's buffered potassium iodide solution. No free ozone was detected by either the rubber-cracking test or bio-assay.

Twenty-four uniform Zutano avocado seedlings were divided randomly into two groups, one of which was fumigated with the synthetic smog while the other received only clean air. The seedlings were six weeks old and about twelve inches tall when they were transferred from the seed bed into three-gallon glazed crocks filled with sand. The fleshy cotyledons were removed from the seedlings when they were transplanted, to make them dependent upon photosynthesis instead of upon a stored food supply. Hoagland's nutrient solution was applied uniformly once a week, and the sand was flushed with tap water between applications of nutrient solution.

Successive daily fumigations were run for eight weeks, from April 15 until June 10, after which the seedlings were harvested. They had been fumigated intermittently with synthetic smog for a total of 280 hours. The following measurements of growth of the seedlings were made: (a) increase in stem elongation; (b) stem diameter at the base of the new growth and at the base of the seedlings; (c) length and width of new leaves (those produced during the fumigation period); (d) fresh weight and dry weight of new stem, old stem, root system, new leaves, and old leaves (those produced before fumigations started). The seedlings were examined regularly for visible symptoms of leaf injury that might be attributed to an air-borne phytotoxicant.

## RESULTS

After ten days, or approximately 70 hours of fumigation with the synthetic smog, a flecking of brown discoloration was noted only on the lower surface of the newly expanded avocado leaves in the treatment chamber. This discoloration became more noticeable as the fumigations continued, but it did not appear on all seedlings in the treatment chamber or on the mature and the very young leaves of any one seedlings. A necrosis of the tip and margin of some leaves developed on about one-third of the fumigated seedlings (Fig. 1) but not on the control seedlings.

Linear measurements of fumigated and nonfumigated avocado seedlings are presented in Table 1 for comparison. The average stem elongation of the fumigated seedlings was 56 per cent less than that of control seedlings grown in clean air. All the seedlings grew continuously throughout the experiment; the difference in stem elongation was the result of the short internodes produced by the fumigated seedlings. The fumigated seedlings produced an average of 11.2 new leaves per seedling, compared with an average of 11.7 new leaves produced by the seedlings grown in clear air.

*Fig. 1.* Zutano avocado seedling showing necrosis of leaf tips and margins after fumigation with reaction products of ozone and 1–N–hexene vapor (synthetic smog).

New avocado leaves produced in the presence of clean air averaged 35 per cent larger than did leaves produced by seedlings fumigated with synthetic smog (Fig. 2, Table 1). Differences in new leaf area were not apparent during the early part of the fumigation period but became progressively more pronounced near the end of the eight-week fumigation period. The general leaf shape was not altered, the ratio of length to width remaining identical for fumigated and nonfumigated seedlings. The new avocado leaves became progressively more chlorotic as the fumigations continued and appeared somewhat rugose in comparison with leaves of nonfumigated seedlings.

*Table 1.*—Effect of fumigation with the reaction products of ozone and 1–N–hexene vapor (synthetic smog) on growth of Zutano avocado seedlings, 1956.

| Plant part | Plants measured | | L.S.D. (1%) |
|---|---|---|---|
| | Fumigated | Nonfumigated | |
| | *mm.* | *mm.* | |
| Stem length (increase) | 40.0 | 92.0 | 25.0 |
| New stem (diameter) | 3.0 | 5.0 | 0.2 |
| Old stems (diameter) | 5.5 | 7.0 | 0.9 |
| New leaves (width) | 43.0 | 66.0 | 7.4 |
| New leaves (length) | 111.0 | 172.0 | 23.0 |

The effect of the fumigations upon the fresh weight and dry weight of avocado seedlings is shown in Table 2. The average fresh weight of the fumigated seedlings was 53.6 per cent less than that of control seedlings. The old leaves produced before the fumigations started were the only parts of the seedlings in which the fresh weight was not reduced significantly by fumigation. However, the dry weight of the old leaves from the fumigated seedlings was reduced significantly compared with that of the control seedlings, Table 2. The reduction in dry weight of all parts of the fumigated seedlings, except the old leaves, was significant at the one per cent level.

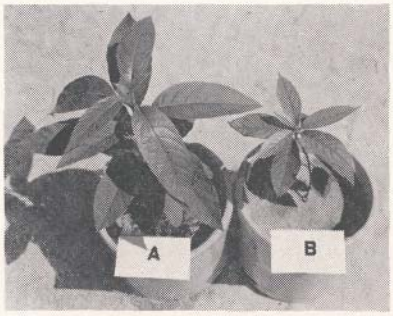


*Fig. 2.* Effect of fumigation with reaction products of ozone and 1–N–hexene on growth of Zutano avocado seedlings. A. Tree grown in clean air filtered through activated carbon. B. Tree grown in clean air but fumigated daily with reacted ozone and hexene.

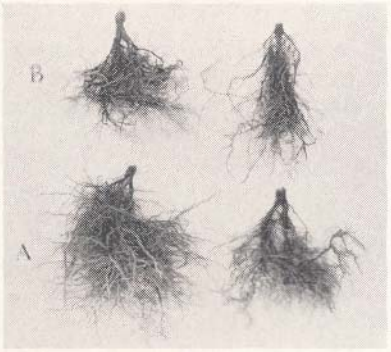


*Fig. 3.* Effect of fumigation with reaction products of ozone and 1–N–hexene vapor (synthetic smog) on volume of root systems of Zutano avocado seedlings. A. Roots of seedlings grown in clean air. B. Roots of seedlings grown in clean air but fumigated daily with controlled concentrations of ozone and hexene vapor.

*Table 2.*—Effect of fumigation with the reaction products of ozone and 1–N–hexene (synthetic smog) on average fresh and dry weights of pairs of Zutano avocado seedlings, 1956.

| Plant part weighed | Average fresh weight per plant | | | Average dry weight per plant | | |
|---|---|---|---|---|---|---|
| | Fumigated | Non-fumigated | L.S.D. (1%) | Fumigated | Non-fumigated | L.S.D. (1%) |
| | *gm.* | *gm.* | | *gm.* | *gm.* | |
| New stem | 0.5 | 2.2 | 0.7 | 0.14 | 0.37 | 0.06 |
| Old stem | 6.6 | 10.2 | 3.0 | 1.4 | 3.4 | 1.11 |
| New leaf | 5.6 | 14.6 | 3.4 | 1.1 | 3.9 | 0.98 |
| Old leaf | 8.2 | 8.7 | N.S. | 2.3 | 3.1 | 0.32[a] |
| Roots | 16.3 | 44.7 | 13.2 | 1.9 | 5.5 | 1.8 |
| Total | 37.2 | 80.2 | 6.1 | 6.84 | 16.27 | 4.1 |

[a]L.S.D. at 5% level.

The total volume of roots produced by the avocado seedlings grown in the presence of the synthetic smog was greatly reduced (Fig. 3). Many of the small feeder roots on the fumigated seedlings were dead, and new root growth was less vigorous than was that of control seedlings grown in clean air. The average fresh weight and dry weight of roots from the fumigated seedlings was reduced 63.5 per cent and 65.5 per cent, respectively, compared with control seedlings (Table 2).

## DISCUSSION

Growth of Zutano avocado seedlings was greatly suppressed and leaf injury was produced by repeated exposure to the reaction products of ozone and 1-N-hexene vapor (synthetic smog). This is in contrast to the report by Todd *et al.,* (9) and to previous attempts by the authors to induce leaf injury by short exposures of 14 hours or less to a series of concentrations of reacted ozone and 1-N-hexene. A particular

conditioning of avocado leaf tissue appears to be necessary before visible injury will occur, since only leaves in the process of expanding were injured. Length, of exposure also appears to be a limiting factor, since injury was observed on the young leaves only after about 70 hours of fumigation.

The leaf injury, a mottled brown discoloration on the lower leaf surface, may be clue to desiccation caused by increased permeability of leaf cells exposed to oxidants formed by reacting o/.one with 1-N-hexene vapor (synthetic smog). Wedding and Erickson (10) found that synthetic smog, the reaction products of ozone and hexene, resulted in an initial decrease in the permeability of the cells of pinto bean leaves, but that fumigations of over three hours resulted in a reversal of this effect and increased the permeability of the leaf cells. This increased permeability was associated with the appearance of visible smog damage on the bean leaves. The marginal necrosis of leaves on one third of the avocado seedlings was perhaps a result of a genetic characteristic, avocados being heterozygous, which intensified the increase in cell permeability induced by the synthetic smog.

The suppression in growth of Zutano avocado seedlings was probably due to a deficiency of available synthesized carbohydrates for proper expansion and maturation of the new tissue, rather than to direct toxicity to meristematic tissue. The fact that exposure of the avocado seedlings to the synthetic smog resulted in a greater reduction in dry weight than in fresh weight of the new leaves and stems tends to support this theory. The fresh weight of old leaves (those fully expanded at the beginning of the experiment) was not affected significantly by the reacted ozone and hexene, but the dry-matter content was reduced significantly.

The deficiency in available carbohydrates may be due to increased respiration, as suggested by Todd (8) on lemons, and by Erickson and Wedding (2) for *Lemna minor,* or to a reduction in photosynthesis, as shown by Erickson and Wedding (2). Erickson and Wedding also reported a 50 per cent reduction in chlorophyll content of *Lemna minor* plants exposed to ozonated hexene for 24 hours. A similar destruction of chlorophyll may account for the progressive increase in the chlorosis of young avocado leaves during the 280 hours of fumigation with the reaction products of ozone and 1-N-hexene vapors (synthetic smog).

The composition of natural smog is no doubt much more complex than that of the synthetic smog used in this experiment. Therefore, experiments arc in progress to determine possible growth effects of natural smog and to relate these results with the growth suppression produced by synthetic smog.

## SUMMARY

Zutano avocado seedlings were fumigated with the reaction products of ozone and 1-N-hexene vapor (synthetic smog) for 280 hours over a period of eight weeks to determine the effect, on growth. Fumigation with this synthetic smog reduced stein elongation 56 per cent; leaf width, 35 per cent; leaf length, 35 per cent; trunk diameter, 21 per cent; fresh weight of seedlings (including the root system), 52 per cent; dry weight of seedlings (including the root system), 58 per cent. A characteristic brown discoloration was observed on the lower surface of young expanding leaves after about 70 hours exposure to the synthetic smog. Marginal and tip necrosis of leaves of all ages occurred

on some of the fumigated avocado seedlings. No leaf injury was observed on avocado seedlings grown in clean air.

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