Ozone: Depression of Frond Multiplication and Floral Production in Duckweed

Abstract. *Plants of* Lemna perpusilla *#6746 grown in an environment charged daily with a low concentration of ozone over 2 weeks were slower to begin multiplying, had a significantly lower rate of frond doubling, and required longer to produce fewer flowers than control plants. Treated plants produced smaller, slightly yellow fronds but had no symptoms of acute injury. Control plants produced four times as many fronds and six times as many flowers as plants continuously exposed to ozone (0.1 part per million).*

Short-term effects of photochemical (oxidant) air pollutants on plants and plant growth have been studied (*1*). However, little is known about long-term effects of periodical pollution on the development of plant populations (*2*). We report such effects on development of a population of the aquatic plant *Lemna perpusilla* strain #6746 (*3*) exposed daily to ozone (0.1 ppm) in a controlled environment.

A clone of *L. perpusilla* #6746 was allowed to multiply aseptically on one-third strength Hutner's medium (*4*) in 2-liter Fernbach flasks under continuous light of 350 to 400 lu/m² at 26°C for 2 weeks. A number of uniformly sized, mature plants consisting of a parent frond and a single "right hand" daughter frond were selected for study. Five plants were randomly placed in each of several standard plastic sterile petri dishes filled with 10 ml of medium. Five plants were used per dish to reduce the crowding effects and to facilitate handling and counting the plants.

The plants were grown at 26°C under 350 lu/m² of cool white fluorescent light on an 8 hour light, 16 hour dark regime to promote flowering (*5*). For 2 weeks the dishes were opened daily for counting. Each day after counting, plants were then transferred aseptically to other Petri dishes in two plexiglass chambers, for exposure to ozone or to fresh scrubbed air. These petri dishes contained fresh medium. The chambers were illuminated with 350 lu/m² and kept at 26°C during fumigation. The fronds in the ozone chamber were exposed for 5 hours per day for 14 days to ozone (0.1 ppm measured coulombmetrically by the Mast Meter) flowing through the chamber at the rate of 1 liter per minute. The controls were exposed to scrubbed air at the same temperature, under the same light conditions and at the same flow rate. The chamber is described elsewhere (*6*). After exposure, the plants were lifted from the chamber dishes and placed in their original holding dishes. These contained the original medium upon which the plants had been grown before treatment. Fresh medium and new dishes were used for each day's exposure to ozone, and for the control plants exposed daily to scrubbed air. This system was used to prevent the fronds from being exposed to possible chemical byproducts accumulated during ozone exposure and to insure that the fronds would be exposed to the byproducts normally secreted into the growth medium by rapidly growing *Lemna* plants.

Control plants showed a short lag phase after which frond numbers doubled every 3 days as a linear function of time when plotted on a log scale. Plants growing under conditions of daily ozone exposure exhibited a longer lag phase and frond numbers doubled about every 7 days. The multiplication of ozone-exposed fronds was nonlinear in function (Fig. 1).

The rate of frond doubling of *L. perpusilla* #6746 exposed to low levels of ozone was less than one-half that exhibited by plants growing in an environment free of ozone. The rate of frond doubling in plants exposed to ozone increased to 4 days and became more nearly linear when previously treated fronds were allowed to develop for 8 days more in an environment without ozone.

Floral production (Fig. 2) followed a pattern similar to frond multiplication. Flowering occurred on the 11th day in control plants and increased linearly thereafter. Flowering among

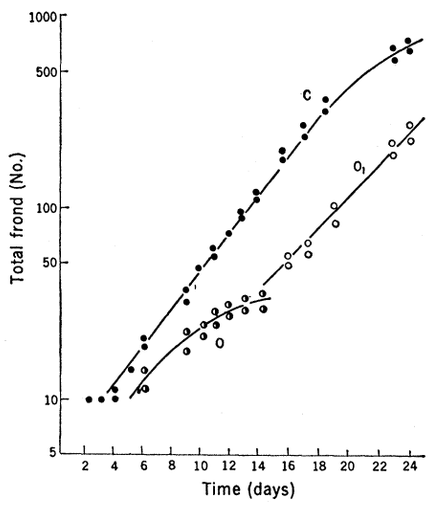

Fig. 1. Repression of frond multiplication of *Lemna perpusilla* #6746 exposed to ozone (0.1 ppm) for 5 hours per day for 14 days. Total numbers of fronds are plotted against days elapsed on a logarithmic scale. *C*, control; *O*, exposed to ozone; O_1, ozonated fronds allowed to grow in ozone-free environment. Curve *C* shows linear response during logarithmic portion of growth cycle. Curve *O* is nonlinear and the difference between *C* and *O* at 14 days indicates that 100 percent of fronds are affected. Fronds treated the same and grown in ozone-free environment (O_1) again display a linear growth curve whose slope is similar to that of *C*. Curve *O* would appear to be a dosage response curve in which dosage is varied by exposure time. Plotted on arithmetic probability paper *O* appears to be linear. (No ozone exposure after 14 days.)

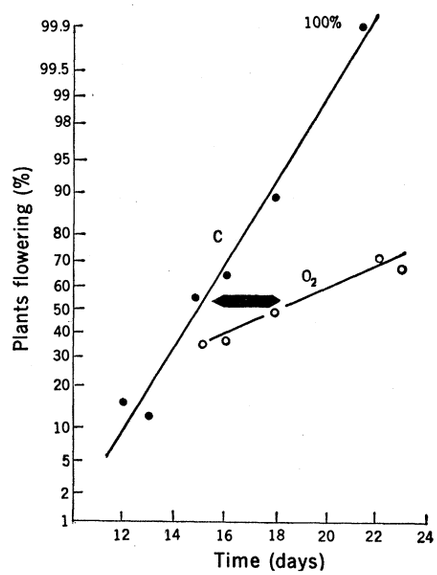

Fig. 2. Repression of floral initiation time and floral development in *Lemna perpusilla* #6746 exposed to ozone (0.1 ppm) for 5 hours per day for 14 days. *C*, control; and O_2, flowers developing on plants exposed to ozone. The number of plants flowering, expressed as a percentage, are plotted against days elapsed in an arithmetic probability plot. Both curves are linear, with differing slopes, indicating that both rate of flowering and proportion of plants flowering are truly different in the two environments. Flowering begins earlier in the ozone-free environment, and the number of flowering plants continues to rise more rapidly in this environment than in the ozone-supplemented environment. The heavy arrow represents the lag in flowering among plants exposed to ozone at the 50 percent level. Note that no flowering occurs among plants exposed to ozone until they are removed from the ozone-supplemented environment. (No ozone exposure after 14 days.)

plants exposed to ozone occurred after 15 days and the number of flowers increased linearly but at about one-half the rate exhibited by the control plants.

After 16 days there were roughly four times as many fronds in the controls as in the exposed plants. There were about six times as many flowers in the untreated plants as in the exposed plants. By the 24th day, after 8 days of ozone-free growth for both plant groups the gap between frond numbers had dropped slightly but the ratio of flower number between control and treated plants remained at about six to one.

Among treated plants, frond size was slightly reduced, and fronds tended to separate more readily when mature. There was a slight degree of yellowing which was not visible in untreated plants but no bleaching or other gross injury ever appeared.

These profound changes in population development and floral production occurred in the presence of amounts of ozone often encountered in the ambient air of the eastern seaboard during summer days. These effects coupled with observations on inhibition of pollen germination and tube elongation (*6*) suggest that low amounts of photochemical oxidants in the air environment may exert a selective effect upon plant populations.

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References and Notes

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Cellular Response to Ecdysterone in vitro

Abstract. *Organ cultures of hindgut from diapausing tobacco hornworm pupae,* Manduca sexta *(Johannson), produce an abundance of migrant cells around the original explant. With time-lapse cinematography, these cells were seen to move slowly and tended to aggregate. The addition of ecdysterone (β-ecdysone) to these cultures stimulated a large increase in individual cell movements and the eventual disruption of cell sheets.*

The hindgut of a diapausing lepidopteran pupa is a narrow tube lined by a simple epithelium that is, in turn, surrounded by a mass of ill-defined tissue, probably composed of larval muscle fibers, deteriorated cryptonephric Malpighian tubules and sheaths, and hemocytes. During adult development, this sheath disappears, and the rectal sac enlarges and fills with waste material. Only a thin muscularis remains around the adult rectum (*1*).

The sequence of morphogenetic events involved in normal adult rectal development (*1*) has been reproduced by injecting molting hormone into diapausing pupae. We have now studied this effect in vitro.

We used diapausing pupae of the tobacco hornworm, *Manduca sexta* (Johannson), which were obtained by exposing the eggs and larvae to a short day photoperiod with 12 hours of light and 12 hours of darkness. After anesthesia with carbon dioxide, the hindgut or posterior portion of the hindgut was removed, rinsed with three changes of culture medium containing a small amount of phenylthiourea to inhibit phenolases, and then placed in a Rose multipurpose culture chamber (*2*). The explanted organ was held against the glass coverslip by a strip of dialysis tubing (*3*). The assembled chamber was injected with Grace's insect tissue culture medium (Grand Island Biological) (*4*) as modified by Yunker *et al.* (*5*), and placed at 27°C; the medium was changed every 2 to 3 weeks.

Two days after they were explanted, single cells began to migrate from the hindguts onto the cover glass. This migration continued for several days or weeks, depending on the condition of the explant, and the migrating cells frequently formed into locally dense sheets around the explant. These migrant cells flattened against the cover glass and extended long cytoplasmic processes that tended to join and form reticuli or contiguous sheets of cells (Fig. 1). When viewed under time-lapse photomicrography, the cells moved with slow, gliding motions, and their cytoplasmic processes exhibited little peripheral activity. Examination of stained sections of explants cultured for several weeks showed that the hindgut epithelium was intact and appeared similar to that of normal diapausing pupae. However, the sheath of tissue normally surrounding the epithelium was considerably reduced in thickness, and the migrant cells appeared to originate from the sheath.

After several weeks in vitro, ten hindgut culture chambers were injected with ecdysterone (Mann Biochemical) dissolved in either sterile distilled water or methanol (nanograde, Mallinckrodt) (2 μg/μl); four control chambers received solvent alone. An additional series of four chambers was injected with 5 to 25 μg of 22-isoecdysone per milliliter (*6*), a compound with no known molt-stimulating properties in *Calliphora* (*7*). Tests on diapausing hornworm pupae whose brains had been removed showed that 22-isoecdysone was unable to induce development at concentrations up to 25 μg per gram of fresh body weight, but ecdysterone at a concentration of 1 μg/g stimulated adult development in similar pupae.

Positive responses in vitro were elicited by ecdysterone at concentrations of 2.5 to 25 μg per milliliter of culture medium as noted by a visible change in the behavior of the cells, whereas the controls remained unaffected.

The cultures were photographed continuously by time-lapse cinemicrography immediately before and for several days after injection. After a delay of from 8 to 20 hours after the addition of hormone, the normal, undulating movements of the membranes of the isolated migrant cells began to increase in speed and frequency, and the random movements of the cells over the coverslip became more rapid. This effect intensified for several hours and spread to the cells forming the dense sheets. Occasional connections sometimes formed between adjacent cells, but the general tendency was toward dissociation. Contiguous sheets of cells eventually dispersed into groups of "excited" single cells (Fig. 2) that retracted their elongate processes and tended to be-